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Protein composition of the cytoskeleton of protists

Proteinové složení cytoskeletu protist

Bachelor thesis

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Declaration

I, Eva Švagr, declare that this thesis has been composed solely by myself and that I have acknowledged all results and quotations from the published work of others. Neither this thesis nor any substantial part of it has been submitted in any previous application for this degree or any other.

Prague, 4. 6. 2020

Acknowledgments

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Abstract

While we have a good understanding of the roles of actin and tubulin filaments in the cell cytoskeleton, intermediate filaments (IFs) are often overlooked. However, the importance of IFs becomes quite apparent, as proteins similar to IF proteins have been identified in many protist cells. This holds particularly for the cells of protists, where even the functions of some members of the of actin and tubulin superfamilies remain unclear. Intermediate filaments are still not well established as components of protist cytoskeletons, in contrast to their more thoroughly studied counterparts in Metazoa. Protist and metazoan IF proteins are dissimilar in their sequence; however, they share similarities in structure, and they assemble autonomously into analogous filaments. IF-like proteins have been localized to striated fibers or unique cytoskeletal components in several organisms, even though not much is known about the protein composition of these striated fibers to date. This suggests that IF-like proteins might be a universally present component of these striated fibers often seen in protist cells.

Keywords: Protists, cytoskeleton, microtubules, microfilaments, intermediate filaments, fibrils, protein composition

Abstrakt

Zatímco máme jasnou představu o roli aktinu a tubulin v buňce, intermediární filamenta (IF) jsou často přehlížená. Tak je tomu především v buňkách protist, kde nejsou známé funkce ani u členů širších rodin aktinu a tubulinu. Intermediární filamenta stále nejsou u protist vnímána jako součást cytoskeletu, narozdíl od jejich lépe prostudovaných protějšků u metazoí. Proteiny intermediálních filament u protist a metazoí si nejsou sekvenčně podobné, nicméně sdílí podobnou strukturu a schopnost autonomně se uspořádávat do analogických filament. Význam intermediálních filament se stal zřejmým, když byly proteiny podobné složkám IF identifikovány v mnoha protistních buňkách. Přesto že o proteinovém složení žíhaných fibril nevíme mnoho, IF proteiny byly u několika organismů lokalizovány právě do žíhaných fibril nebo jiných unikátních cytoskeletálních útvarů. Toto naznačuje, že IF proteiny by mohly být více univerzálně přítomným komponentem filament, které jsou často pozorovány v buňkách protist.

Klíčová slova: Protista, cytoskelet, mikrotubuly, mikrofilamenta, intermediární filamenta, fibrily, proteinové složení

1 Introduction

Possession of a complex cytoskeleton is considered one of the key features of eukaryotic cells. There are three cytoskeletal proteins capable of filament formation: actin that forms the microfilaments (MF), tubulin that forms the microtubules (MT), and the intermediate filaments (IFs) that are composed of a variety of proteins. Each of these filament types is built differently and has its own unique role. Actin and tubulin fulfill roles in mitosis, transport, and motility, by means of microtubule-based flagella or microfilament-based filopodia. Intermediate filaments, on the other hand, are more of a structural and less dynamic component, mainly providing resistance against mechanical stress as well as support for membranes. This leads to very different rates of evolution of the proteins that form them, which is especially seen in the very rapid diversification in IF proteins, and renders them difficult to be identified from the primary sequence. Another important difference between actin, tubulin, and IFs is that, while IFs have only been reported from some groups, the first two are present in all eukaryotic cells.

The consensus presented in both scientific and teaching literature usually identifies IFs as a Metazoa-specific feature. Only recently, homologs of the lamin family, one of the most thoroughly studied components of IFs, were recognized in protists. Most of the knowledge on cytoskeletal proteins we have comes from studies on conventional model organisms, which do not reflect the diversity of eukaryotic organisms, especially when considering cell organization, shape, or motility. This makes IF proteins an understudied enigma, and a point of interest at the same time. Many things are still unclear about IFs, including their presence in the Last Eukaryotic Common Ancestor (LECA) and their evolutionary origin.

This thesis aims to review the knowledge on individual cytoskeletal proteins and the filaments they from in the context of protist cells highlighting their features and differences. Both actin and tubulin superfamilies will be discussed, along with families of IF proteins. Protein composition of some unique protist structures will be reviewed at the end.

2 Tubulin

The tubulin superfamily contains six major families (α, β, γ, δ, ε, ζ), all of which seem to have been present in LECA (Findeisen et al., 2014) (Figure 1). The first to diverge from the ancestral tubulin gene was γ-tubulin followed by the common ancestor of α and β-tubulins. A δ/ζ-tubulin form a clade sister to ε-tubulin (Findeisen et al., 2014). Two additional tubulin groups, θ and ι, have been reported in *Paramecium* sp., however, they stem from β-tubulins. Likewise, α-tubulins include the smaller subgroup of κ-tubulins (Findeisen et al., 2014; Libusová & Dráber, 2006). Prokaryotic homologs are also part of the larger protein superfamily. These homologs include, among others, include FtsZ and TubZ (Findeisen et al., 2014; Wickstead & Gull, 2011). FtsZ is a widely distributed prokaryotic protein that forms contractile ring during cell division (Wickstead & Gull, 2011). TubZ, on the other hand, is involved in plasmid segregation (Larsen et al., 2007).

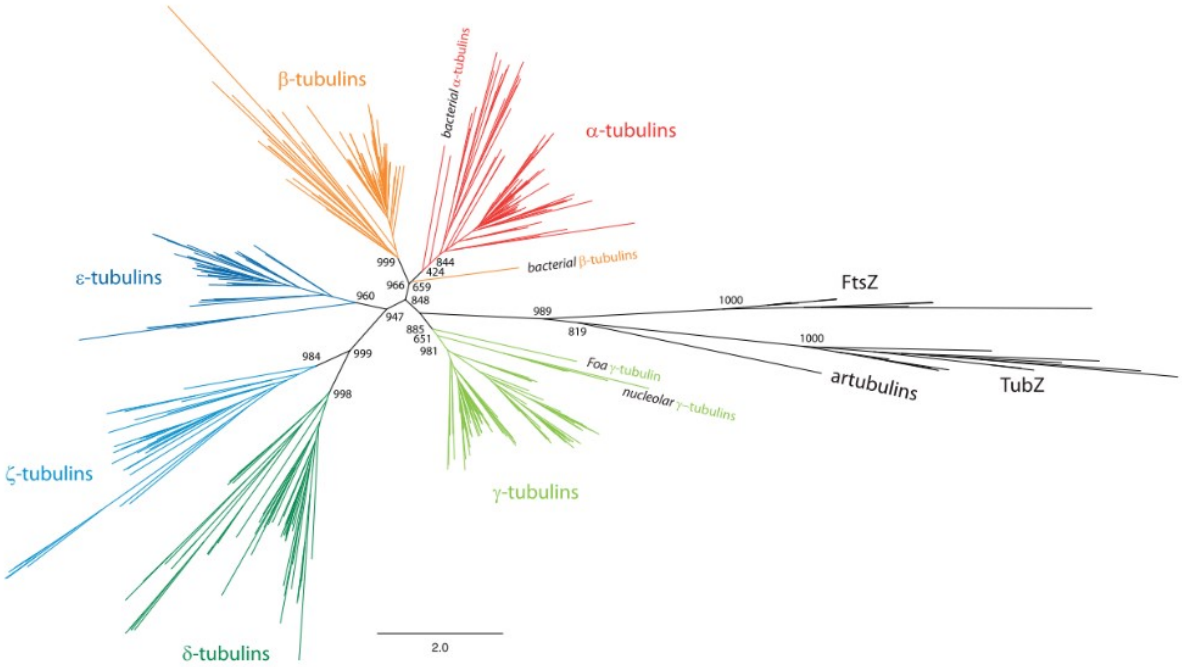


Figure 1 Phylogenetic tree of tubulin superfamily, Numbers stand for maximum-likelihood bootstrap values from 1000 replicates, scale bar represents estimated amino acid substitutions per site (Findeisen et al., 2014).

The α- and β-tubulin dimer is a building block of polarized filaments – the microtubules (Figure 2). The heterodimers are bound together in a head-to-tail manner to form protofilaments, which subsequently give rise to microtubules via lateral association. Tubulin protofilaments have two distinct ends; the (-) end with exposed α-tubulin and the (+) β-tubulin end (Figure 2). Polymerization occurs on both ends, but more rapidly at the (+) end. Out of the two subunits, only β-tubulin can hydrolyze GTP; the presence of this GTP is crucial for the ability of the heterodimer to polymerize at the (+) end. Thus the (+) end bears a cap of GTP bound β-tubulin, while in the rest of the filament β-tubulin is found in the post-hydrolysis GDP-containing form (Desai & Mitchison, 1997). Intriguingly, it has been reported that α- and β-tubulin sometimes takes up a form distinct from the canonical microtubular organization, for example in the conoid of *Toxoplasma gondii* (Hu et al., 2002) or the helical filaments of Foraminifera (Bassen et al., 2016). The microtubular complex has many functions, from cell division to flagellar motility to

intracellular transport. Among structures composed of microfilaments are the flagellar axoneme and the mitotic spindle (Nogales, 2001).

Tubulins show a three-domain structure with an N-terminal GTP binding domain (a nucleotide binding domain - NBD), an intermediate domain that interacts with taxol (a drug that stabilizes microtubules), and a C-terminal domain that forms a crest on the microtubular surface (McKean et al., 2001). Tubulin sequences are very conserved in length (Findeisen et al., 2014). The non-microtubular tubulins express insertions and deletions as compared to α- and β-tubulin; these are usually restricted to loop regions (McKean et al., 2001).

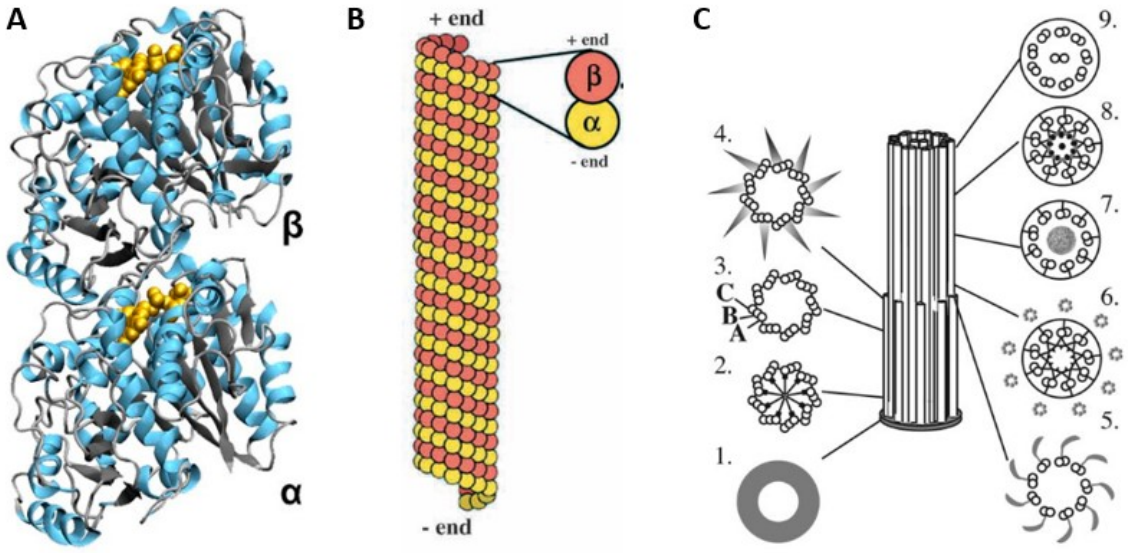


Figure 2 - A) cartoon of α- and β- tubulin dimer, with GTP represented in yellow (Wickstead & Gull, 2011); B) microtubule composed of α- and β- tubulin (Inclán & Nogales, 2001); C) structural organization of the basal body of *Chlamydomonas*. Note the description of complete A tubules and incomplete B/C hemitubules in cross-section number 3 (O'Toole et al., 2003).

Alpha- and beta-tubulins are ubiquitous in Eukaryotes and tend to be present in several isotypes in the cell. They are well conserved, with notable changes limited to C-terminus (McKean et al., 2001). Gamma-tubulin is also ubiquitous and along with the two previously mentioned, represents the minimal tubulin set (Oakley, 2000; Ruiz et al., 1999).

Tubulins δ, ε, and ζ have been found in all major eukaryotic supergroups, but appear to have been independently lost in some lineages (e.g. Dikarya, Spermatophyta, and Diptera) (Figure 3) (Findeisen et al., 2014). Interestingly, their occurrence often correlates with the presence of triplet microtubules in basal bodies and centrioles, which serve as Microtubule Organising Centres (MTOCs), hinting at their function being involved with processes related to basal bodies and centrioles (Dutcher, 2003; Marshall & Rosenbaum, 2003; McKean et al., 2001). In the proposed model, ζ-, ε-, δ- tubulin form an evolutionarily conserved ZED module (named from the Greek letters) and together partake in the orientation and functionalization of the centriole. The ZED module appears to have been lost in several lineages; in particular, it is missing from some fungi, plants, and animals, which do not exhibit the canonical triplet organization (Dutcher, 2001b; Turk et al., 2015). Surprisingly, none of the non-microtubular tubulins have been identified in *Giardia intestinalis*, that (Findeisen et al., 2014).

It seems that the large number of tubulin isoforms contribute to the formation of a variety of different microtubular structures (Dutcher, 2001a). The great variability of the tubulin superfamily lies not only in its numerous subfamilies but also in the post-translational modifications of tubulins' C-termini (Ludueña, 1997). These modifications include phosphorylation, acetylation, palmitoylation, sumoylation, polyamination, S-nitrosylation, tyrosination, glutamylation, and glycylation (Yu et al., 2015). Well-known modifications are acetylation and (de)tyrosination of α-tubulin: both are found on more stable and long-lived, rather than dynamic, microtubules. These modifications function as markers rather than the stabilizing factors (Rosenbaum, 2000; Yu et al., 2015). Modifications of tubulin vary in different microtubular structures, sometimes even between tubules of the same tubulin protofilament (Campanati et al., 2003; Yu et al., 2015). Interaction with other proteins such as molecular motors (kinesin and dynein) or other cytoskeletal components (e.g. vimentin), is dependent on specific posttranslational modification (Rosenbaum, 2000).

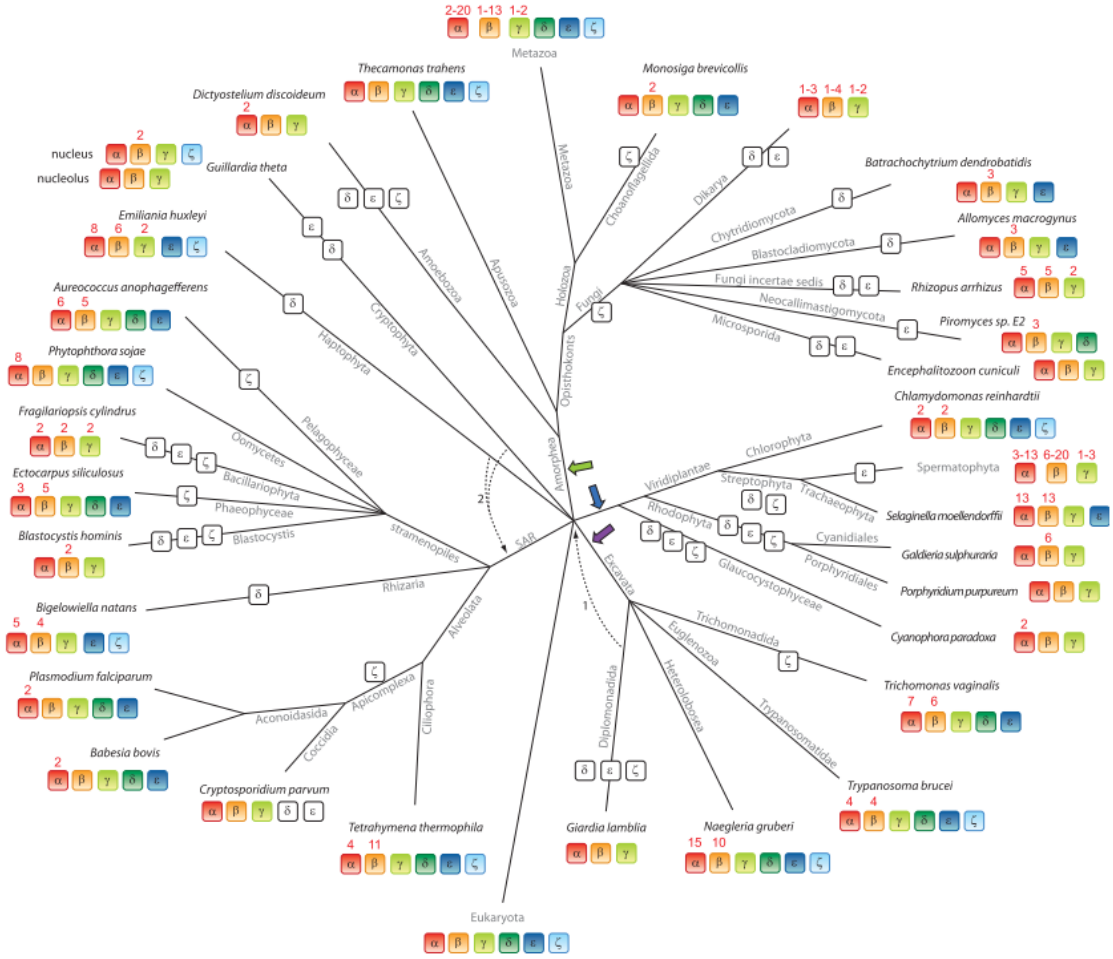


Figure 3 – Distribution of tubulin across the tree of eukaryotes – Losses of tubulin subfamilies are indicated by white squares; red numbers represent duplicates in given taxon or species (Findeisen et al., 2014)

This chapter focues more on the γ-, δ-, ε-, and ζ-tubulins, and in particular on their divergence among protist lineages. In the following sections, a short overview of each of them and its role in protist cells is given.

2.1 Gamma tubulin

Like of α- and β- tubulin, γ-tubulin is an ubiquitous component of eukaryotic cells. It was first described in *Aspergillus nidulans* (Oakley & Oakley, 1989). The main role of this protein is the nucleation of microtubules (Figure 4). Therefore, it can be found in basal bodies, centrioles, and other MTOCs. It is a minor component compared to α- and β- tubulins (Stearns et al., 1991). More divergent γ-tubulins are observed in organisms where other members of other tubulin families are absent (McKean et al., 2001).

A fair amount of conservation is observed, especially in the N-terminal part of the γ-tubulin protein (Scott et al., 1997; Stearns et al., 1991). An intact nucleotide-binding domain is essential for the proper nucleation of basal bodies (Shang et al., 2005). A similar surface with that on β-tubulin is present in regions interacting with the minus end of α-tubulin, likely participating in a similar interaction (Inclán & Nogales, 2001).

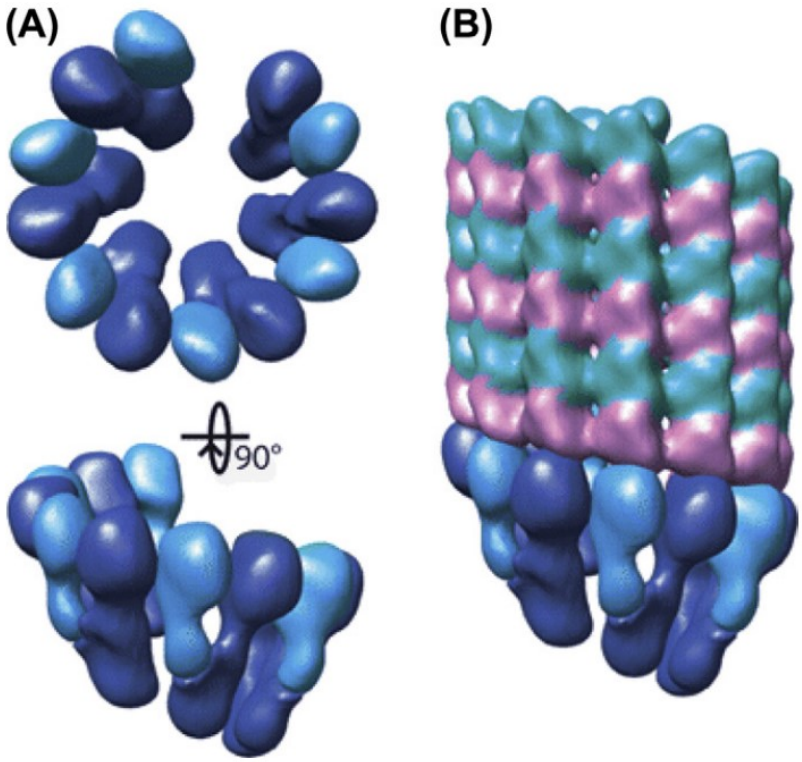


Figure 4 – Model of nucleation of microtubules by γ-tubulin; γ-tubulin represented by shades of blue binds α-tubulin (purple) on its (-) end (Kollman et al., 2008)

Gamma-tubulin's nucleation properties are nicely illustrated by its presence at the bases of newly forming flagella in dividing *Giardia intestinalis* (Nohýnková et al., 2000). *Trypanosoma brucei* γ-tubulin also localizes to basal bodies, to the minus ends of microtubules, and in a region of the nucleus (Scott et al., 1997). In the ciliate *Tetrahymena thermophila,* a single γ-tubulin isoform was present in all basal bodies, small patches associated with macronuclear envelope, particles associated with micronuclear envelope, and two posterior contractile vacuole pores (Shang et al., 2002). A similar distribution was found in another ciliate *Paramecium tetraurelia* (Klotz et al., 2003).

Yet another ciliate *Euplotes focardii* possesses two isotypes of γ-tubulin. One of them localizes to the basal bodies of non-motile cilia, while the other associates only with the centrosome during micronuclear mitosis. The transcription levels of these two γ-tubulin are also different. The most divergent regions of these two tubulins are those that participate in longitudinal and lateral bonding. It is presumed that *E. focardii* γ-tubulin evolved as an adaptation for the psychrophilic conditions that this organism inhabits (Marziale et al., 2008).

Gamma-tubulin was found to be essential in MTOCs of all organisms analyzed (Horio et al., 1991; Pastuglia et al., 2006; Sunkel et al., 1995) including *Paramecium* (Ruiz et al., 1999). The depletion of the protein leads to an inability to duplicate (Ruiz et al., 1999) and maintain mature basal bodies (Shang et al., 2002). Participation in the division of the nucleus has also been observed in *Tetrahymena pyriformis* (Joachimiak et al., 2007), *T. thermophila* (Shang et al., 2002), and *P. tetraurelia* (Klotz et al., 2003).

2.2 Delta tubulin

The family of δ-tubulins seems to be tied to organisms that possess a canonical centriole (Carvalho-Santos et al., 2011). In *Chlamydomonas reinhardtii* it is present in basal bodies and mitotic spindles (Dutcher, 2001b). It might attach longitudinally to α-tubulin at the microtubular minus end or bind basal body-associated proteins (Inclán & Nogales, 2001).

The reported role of δ-tubulin lies in the assembly and maintenance (stability or extension) of C-tubules in centrioles and basal bodies in their nucleation sites (Dutcher & Trabuco, 1998; Garreau de Loubresse et al., 2001) and in the early maturation of basal bodies (Fromherz et al., 2004). The function of δ-tubulin could be substituted by a mutant α-tubulin in the case of a knock-out, leading to the assembly of the C-tubule (Fromherz et al., 2004). Deficiency of this protein leads to the loss of the C-tubule (Figure 5), along with mislocation or loss of basal bodies and altered cell shape in *P. tetraurelia* (Garreau de Loubresse et al., 2001). Loss of A- and B-tubules is also observed, although only rarely (Garreau de Loubresse et al., 2001). In *C. reinhardtii*, loss of flagella in knock-out cells is reported, therefore δ-tubulin might be essential to the formation of flagella (Dutcher & Trabuco, 1998). However, δ-tubulin is not essential for survival, and cells lacking this protein are viable (Dutcher & Trabuco, 1998).

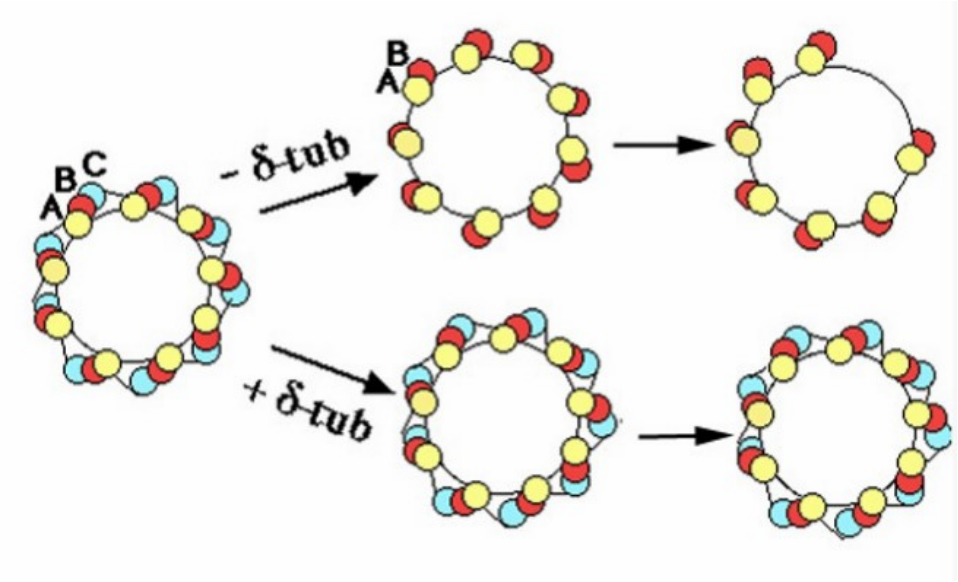


Figure 5 – Illustration demonstrating loss of C-tubule in the case of δ-tubulin depletion (Garreau de Loubresse et al., 2001).

2.3 Epsilon tubulin

Distribution of ε-tubulin is similar to that of δ-tubulin: it is not found in organisms lacking centrioles, e.g., *Arabidopsis thaliana, Caenorhabditis elegans, Saccharomyces cerevisiae* (Carvalho-Santos et al., 2011; Dupuis-Williams et al., 2002). Localization of ε-tubulin is cell-cycle-specific: it localizes only in mature centrosomes, possibly being involved in their maturation (Chang & Stearns, 2000). Antibodies against ε-tubulin label two regions in the basal body apparatus of *C. reinhardtii*; the cage surrounding mature basal bodies and the fibers running along microtubular rootlets (Figure 6) (Dutcher et al., 2002). In cells of *P. tetraurelia*, a similar pattern is observed: even distribution along the microtubule triplets, and in pericentriolar material (Dupuis-Williams et al., 2002). The presence around centrioles might represent a pool of recruitable proteins or an anchoring of the triplets (Dupuis-Williams et al., 2002).

The function of ε-tubulin lies in the stabilization and assembly of the canonical centriole. This protein was shown to be essential to *P. tetraurelia*, *C. reinhardtii*, and *T. thermophila* (Dupuis-Williams et al., 2002; Dutcher et al., 2002; Ross et al., 2013). When silenced, the loss of B- and C-tubules occurs (Figure 6), ultimately resulting in the inability to duplicate basal bodies, presumably after depletion of the available protein (Dupuis-Williams et al., 2002). A role in linking C-and B-tubules is likely (Dupuis-Williams et al., 2002). Its minus end is similar to α- and β-tubulin minus ends; therefore, interaction with the plus end of the microtubule has been proposed (Inclán & Nogales, 2001). Binding GTP into nucleotide binding domains seems to regulate ε-tubulin dynamics (Ross et al., 2013). Epsilon-tubulin is also likely to be subjected to post-translational modifications (Dupuis-Williams et al., 2002).

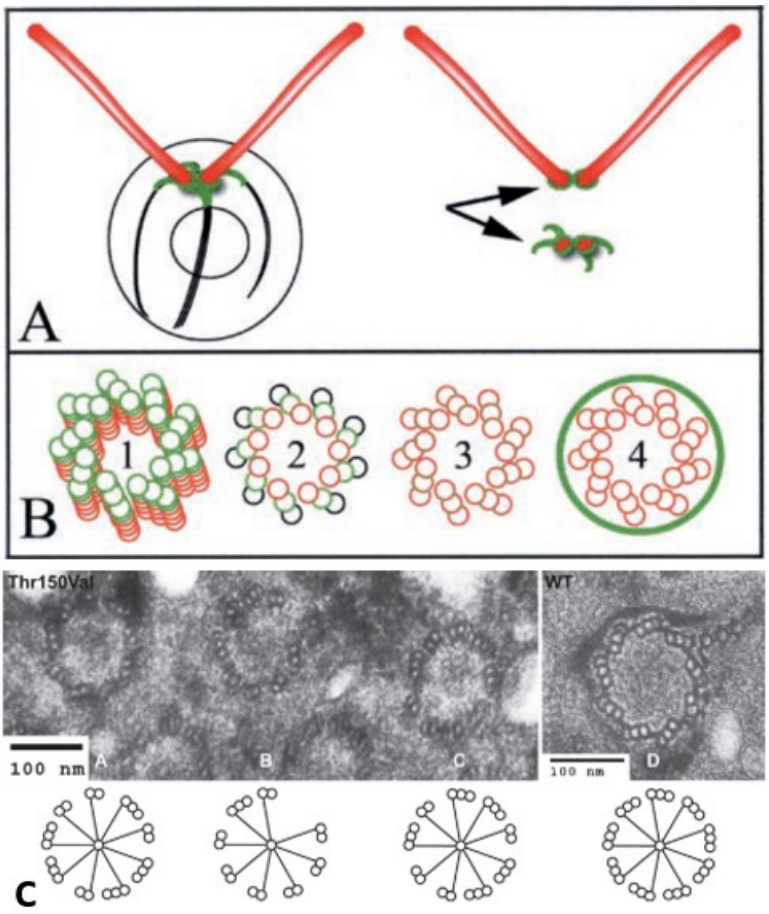


Figure 6 – A) localization of ε-tubulin (green) in whole cells of *C. reinhardtii*; B) models of possible interaction of ε-tubulin (green) in the basal body (Dutcher et al., 2002); C) basal bodies with incomplete microtubular triplets in cells devoid of ε-tubulin (a-c) compared to wild-type basal body (d) (Ross et al., 2013).

2.4 Zeta tubulin

This group was previously termed η-tubulin, but ζ-tubulin has been preferred more recently (Findeisen et al., 2014). It was first described in *Paramecium tetraurelia*, with a proposed role in tethering γ-tubulin to basal bodies (Ruiz et al., 2000). Other suggested functions include capping the minus end of microtubules, lateral interaction with other tubulins, and basal body duplication (Ruiz et al., 2004). A similarity in function to γ-tubulin has been proposed (Turk et al., 2015). Interestingly its presence is often accompanied by δ-tubulin in centriole-bearing organisms (Vaughan et al., 2000).

3 Actin

Actin is a ubiquitous and abundant protein present in all eukaryotic cells. It forms polarized filaments (microfilaments) from two protofilaments coiled together into a right-handed helix. MFs bear two distinct ends: pointed (-) and barbed (+). ATP-bound monomers polymerize on the barbed (+) end, and after ATP hydrolysis, depolymerize from the pointed (–) end in ADP form. This process is called treadmilling observed *in vitro* (see Figure 7). *In vivo*, actin binds to a plethora of proteins that influence its filament-forming by means of capping, binding of monomers, crosslinking the filaments, and stabilizating the filaments. Two forms of actin are present in the cell, G-actin, and F-actin, which are identical but represent the monomeric and filamentous portions, respectively. G- and F-actin also differ in their ATPase activity, which is higher in F-actin and is activated shortly after polymerization. Filaments formed by ATP-bound actin are more stable as opposed to those formed by ADP-bound actin (Dominguez & Holmes, 2011).

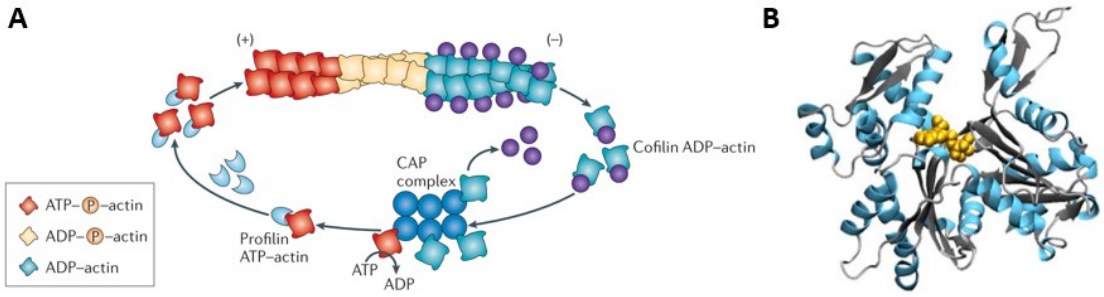


Figure 7 - A) illustration of actin treadmilling showing stages of ATP hydrolysis throughout actin filament (Baum et al., 2006); B) 3D structure of actin monomer (G-actin), with nucleotide shown in yellow (Wickstead & Gull, 2011).

Actin filaments participate in vesicle transport, mitosis, maintenance of cells shape and polarity, migration, and chromatin remodeling (Dominguez & Holmes, 2011). Actin evolution is constrained due to its interaction with a large number of other proteins and systems, resulting in the high level of conservation we see in eukaryotes today (Erickson, 2007). The variable regions usually include parts known to interact with actin-binding proteins and are located on the N-terminus (Bhattacharya & Ehlting, 1995).

Actin belongs in a large family of proteins that includes not only actin related proteins (ARPs), but also the homologous prokaryotic proteins MreB and ParM, HSc70, hexokinase B or glycerol kinase (Dominguez & Holmes, 2011; Kabsch & Holmes, 1995; Wickstead & Gull, 2011). The prokaryotic proteins form filaments similar to F-actin. MreB participates in cell shape maintenance (Jones et al., 2001). ParM is, on the other hand, involved in the segregation of plasmids to the cell poles (Salje & Löwe, 2008). All these proteins share a domain called the actin fold which associates, with either ADP or ATP. The actin fold persists despite great sequence diversity (Kabsch & Holmes, 1995).

In the following text, actin-related proteins are discussed, and afterward a short overview of protist actin variability is given.

3.1 Actin Related Proteins

Actin related proteins are an extended family (Figure 8) with clear sequence similarity to actin but different functions (Frankel & Mooseker, 1996). They likely originated from actin via ancient gene duplications (Bhattacharya & Ehlting, 1995), and most classes were probably present in LECA (Frankel & Mooseker, 1996). ARP classes are characterized by their patterns of indels (Frankel & Mooseker, 1996; Wade et al., 2009). Insertions are usually limited to surface loops, allowing for class-specific interactions while not disrupting the core structure (Wade et al., 2009). One suggested minimal set of ARPs comprises Arp1, Arp4, and Arp6 (Muller et al., 2005).

There are two groups of ARPs – cytoplasmic (Arp1, 2, 3, and 10) and nuclear (Arp4, 5, 6, 8)(Goodson & Hawse, 2002; Muller et al., 2005). The importance of nuclear ARPs is deduced from their presence in a vast diversity of organisms, out of which Arp4 appears to be ubiquitous (Muller et al., 2005). Nuclear ARPs are more diverse than the more conserved cytoplasmic ones (Wade et al., 2009). It is hypothesized nuclear ARPs function in roles once facilitated by ancestral actin and acquired their roles by subfunctionalization (Blessing et al., 2004).

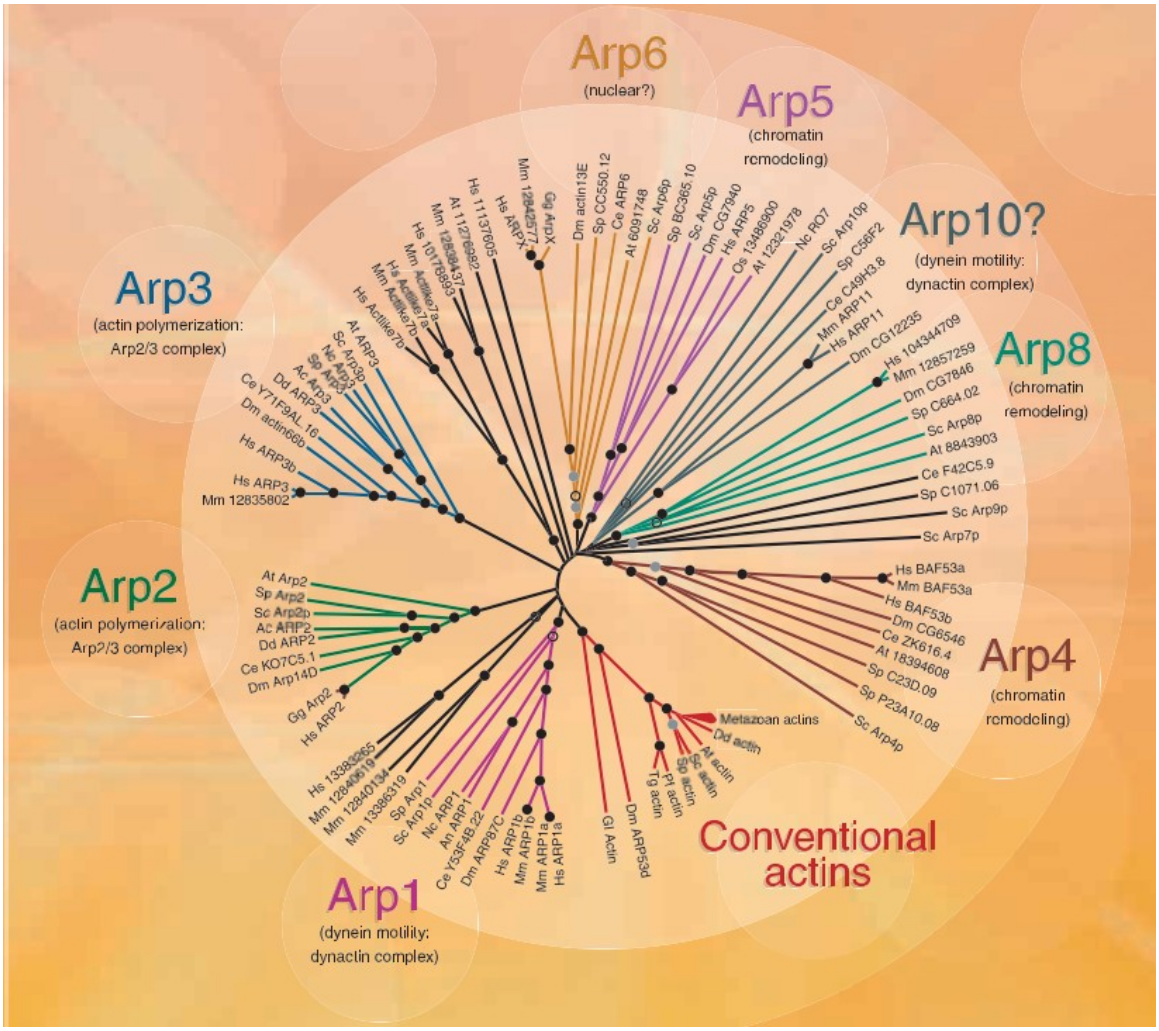


Figure 8 - A phylogenetic tree of eukaryotic actin and ARPs; black, blue, and empty dots represent bootstrap support for individual nodes: strong support (>90%), moderate support (>75%), and suggestive (>50%), respectively. The support for the Arp10 family is weak and therefore their position remains unclear (Goodson & Hawse, 2002).

Some ARP functions are related to actin filaments, for example, nucleation of microfilaments by Arp2 and Arp3 heterodimers (Frankel & Mooseker, 1996; Pollard et al., 2000). Arp1 seems to be capable of polymerization and along with Arp11, it is a part of the dynactin complex (Frankel & Mooseker, 1996), which participates in microtubule-based trafficking (Muller et al., 2005). ARPs 1 through 3 are capable of binding ATP, hydrolysis of which is likely involved in conformational changes of these proteins (Muller et al., 2005). The vast range of functions of Arp4 and its omnipresence suggest that it is a possible ancestor to other nuclear ARPs. Arp4 participates in the following processes: chromatin remodeling, transcriptional activation, DNA repair, apoptosis, tumor suppression, histone acetylation, kinetochore spindle attachment, and gene silencing at centromeres. The suggested role of Arp6 is linked to chromatin remodeling as well (Muller et al., 2005).

3.2 Actin variability in eukaryotes

LECA likely possessed one copy of conventional actin, although a loss of a duplicated actin before eukaryotic radiation cannot be ruled out (Bhattacharya & Ehlting, 1995). Actin genes show a different pattern of evolution in different lineages across the eukaryotic tree, leading to their use for deciphering common trends of gene evolution in those lineages (Goodson & Hawse, 2002; Wade et al., 2009). Likewise, we can see specific sequence patterns in actins of related species. For example, alveolates carry a specific insertion and a conserved asparagine residue (Wade et al., 2009). Some lineages (e.g. fungi or Chlorophyceae) appear to possess only a single copy of actin (Bhattacharya & Ehlting, 1995; Dawson & Paredez, 2013). Red algae (Wu et al., 2009), Ciliata (Yi et al., 2015), Foraminifera (Flakowski et al., 2006), and Dinoflagellata (Kim et al., 2011), on the other hand, possess many actin paralogs, as actin genes are often subject to duplications in these groups.

Actin genes of *Dinophysis* species follow the "birth and death" model, where duplicated genes start to vary and after some time can lose their function and become pseudogenes (Kim et al., 2011). In *Emiliania huxleyi*, we see multiple actin genes, multiple duplications occurred in one particular locus, while other pralogs of actin are dispersed throughout the genome (Bhattacharya & Ehlting, 1995). Actin genes in the genus *Arcella* appear in numerous recent duplications, which differ mostly by synonymous substitutions and remain fairly conserved. Interestingly, *Arcella* species have two loci with actin genes, each of which seems to be influenced by a different selection pressure and has a different duplication rate (Lahr et al., 2011). *Dictyostelium discoideum* has an almost full-range of ARPs (similar to Metazoa) missing only fungal Arp7, Arp9, and Arp10 (Muller et al., 2005).

3.2.1 Ciliata

Ciliate actins have undergone at least three duplications and have been subject to further lineage-specific duplications (Yi et al., 2015). Actins of ciliates are highly divergent from the rest of canonical actins (Croft et al., 2003; Kim et al., 2004; Villalobo et al., 2001; Yi et al., 2015). In *Paramecium tetraureliia*, different paralogues displayed distinct localization, associateing with food vacuoles, the cortex, parts of the oral apparatus, the cytosol, the cleavage furrow, cilia, or the Golgi apparatus. Silencing of some of these paralogues leads to abnormal swimming patterns, altered shape, or impaired phagocytosis (Sehring, Reiner, et al., 2007). The length of these actin

proteins can also diverge from the common length of around 375aa (Sehring, Mansfeld, et al., 2007). Over 30 actin, actin-like, or ARP genes are present in the genome of *P. tetraurelia*, among them several isoforms of ARPs 1-4 (Sehring, Mansfeld, et al., 2007). This divergence might lead mainly to altered interaction with actin-binding proteins (Sehring, Mansfeld, et al., 2007).

3.2.2 Apicomplexa

Interestingly, the actin of *Toxoplasma gondii and Plasmodium falciparum* does not form long filaments as seen in other organisms, but only filaments about 100 nm in length (Schmitz et al., 2005; Schüler et al., 2005b; Schüler & Matuschewski, 2006). *P. falciparum's* actin sequence is divergent particularly in the site responsible for actin-actin interactions within a filament, which might impair their polymerization abilities (Schmitz et al., 2005). Overall, Apicomplexa often possess only a single conventional actin gene along with 8-10 ARPs and several actin-like proteins (ALPs). An exception is *P. falciparum*, in which two homologs of conventional actin have been identified. Described ARPs include homologs of Arp1, 4, and 6. A portion of the ALPs are likely novel for this group and might function in actin-based motility, altough their relationship to ARPs is unclear (Gordon & Sibley, 2005). Actin depolymerizing factor (ADF) is likely one of the main means of maintaining the equilibrium between G-actin and short F-actin filaments in *T. gondii* and *P. falciparum* (Mehta & Sibley, 2010; Schüler et al., 2005a).

3.2.3 Excavata

The diversity of excavate groups is reflected as well in the variety of actin and ARP homologs they possess. While *Trypanosoma brucei* and *Giardia intestinalis* have one conventional actin each (accompanied by 7 and 3 ARPs, respectively), *Naegleria gruberi* and *Trichomonas vaginalis* have multiple variants (29 actins with 49 ARPs, and 12 actins with 11 ARPs, respectively) (Dawson & Paredez, 2013). Actin seems to play a role in the flagella of *G. intestinalis* and *Leishmania donovani* (Paredez et al., 2011; Sahasrabuddhe et al., 2004). *L. donovani* actins are highly divergent from yeast, mammalian, and *Plasmodium* actins, mainly in regions participating in subunit interaction during oligomerization (Sahasrabuddhe et al., 2004). In *Monocercomonoides exilis* six conventional actin genes were identified along with homologs of Arp2, Arp3, Arp4, and Arp6 (Karnkowska et al., 2019).

4 Intermediate filaments and IF-like proteins

While actin and tubulin are well-conserved across the tree of eukaryotes, and their ancestor proteins were present in LECA (Wickstead & Gull, 2011), intermediate filaments were for a long time presumed to be specific to metazoan cells (Peter & Stick, 2015). Nevertheless, proteins with very similar structures, properties, and even functions have been described in many protist lineages, albeit not fulfilling the definition of metazoan IFs. Therefore use of the term "IF-like proteins" is favored (Preisner et al., 2018). Since IF-like proteins have been identified in many groups, it is possible to consider them to be a ubiquitous parts of the eukaryotic cytoskeleton (Preisner et al., 2018). Metazoan IFs are divided into six types: types I and II comprise of keratins, type III includes desmin and vimetin, type IV includes neurofilament protein, lamins belong to type V, and type VI includes nestin (Fuchs & Weber, 1994; Kollmar, 2015). These types are sometimes characterized by a precise organization of their rod domain up to individual repetitive regions and their linkers (Fuchs & Weber, 1994; Herrmann et al., 2009). This might bias the description of other proteins as related, even though they possess the set of common features described below (Preisner et al., 2018).

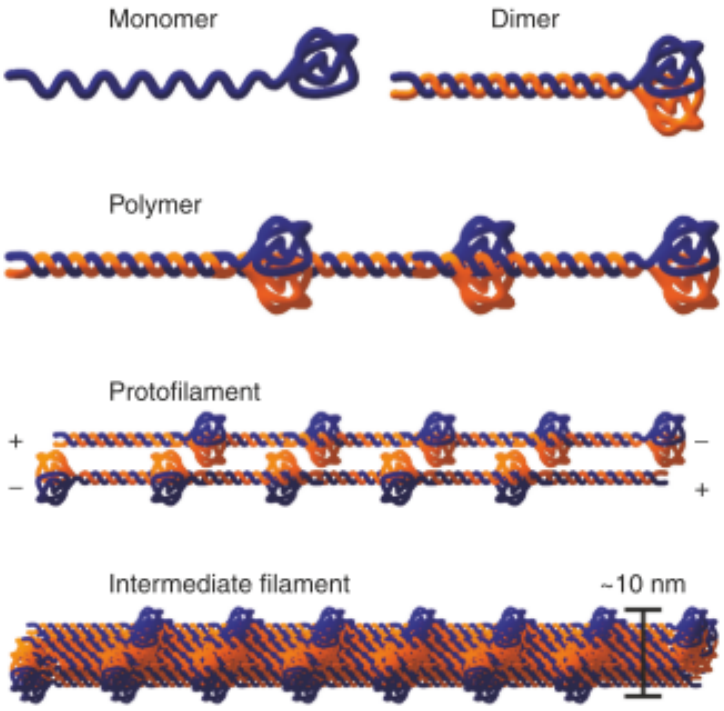


Figure 9- Assembly of IF filaments demonstrated on lamin fibres – first coiled-coil dimer is formed, which polymerizes and dimerizes with antiparallel filament with an overlap, resulting in a protofilament. Three to four ptotofilaments form final 10nm filament (Dittmer & Misteli, 2011).

There is no satisfactory definition for IF-like proteins to date. The most accurate definition would need to be based on their common features, rather than their primary sequences (Herrmann et al., 2009; Peter & Stick, 2015). These include the autonomous formation of filaments (Fuchs & Weber, 1994), resistance to extraction by non-ionic detergents and high-salt-content buffers (Herrmann et al., 2009), and a tripartite structure composed of central α-helical rod and non-helical N- (head) and C-terminal (tail) domains (Fuchs & Weber, 1994). The α-helical rod forms

coiled-coil dimers, which are generally, but not always, longer than what we see in other proteins with these motifs (e.g. myosin) (Rose et al., 2005). The coils are formed as both hetero- and homo-dimers. These later assemble into unpolarized protofilaments, which form the final intermediate filaments (Figure 9) (Fuchs & Weber, 1994; Strelkov et al., 2003). The coiled-coil forming ability is attributed to low complexity repetitive regions, most frequently composed of heptad repeats with hydrophobic amino acids in the first and fourth positions, while polar residues often occupy the fifth and seventh positions (Mason & Arndt, 2004).

IF-like proteins are often grouped together based on the similarity of their most conserved part; the repetitive coiled-coil domain (Preisner et al., 2018). For example, as charged repeat motif proteins, characterized by the presence of the charged repetitive motifs, with an abundance of amino acids: lysine, glutamate, glutamine; leucine; isoleucine; valine. Many of the proteins described below (e.g. alveolins and epiplasmins) would fall into this category along with others such as viral A-like proteins and molecular motors (Gould et al., 2011).

IF-like proteins seem to follow a different evolutionary path than actin and tubulin, and their resemblance seems to stem from convergence rather than divergence from one common ancestor (Preisner et al., 2018). The stark contrast between the evolution of IFs and the components of the two other major cytoskeletal families is hypothesized to be due to the number of their interaction partners. While the main function of IFs is resistance against mechanical stress (Fuchs & Weber, 1994); actins and tubulins fulfill many roles, among them trafficking of material within the cell, and thus they have considerably more interaction partners. Therefore, mutations of these proteins highly influence their functions, consequently constraining the evolution (Fleury-Aubusson, 2003; Preisner et al., 2018). In the case of IF-like proteins, a diversification by gene multiplication with constrained evolution of repetitive regions is observed. This conservation is observed in the length, amino acid composition, number of repeats, and level of sequence similarity among repeats (Gould et al., 2011).

This chapter is dedicated to a description of various IF-like proteins, comparing their similarities, differences, functions, and localization in various protist cells. While the list is extensive, it is not complete, due to the problematic identification of IF-like proteins and the lack of data available in this field.

4.1 Lamins

For a long time, lamins were presumed to be Metazoa specific (Dittmer & Misteli, 2011; Melcer et al., 2007), regardless of previous observations of structures similar to nuclear lamina in protists, such as. *Euglena gracilis*, *Trichomonas vaginalis* and *Giardia intestinalis* (Wen, 2000; Wen & Li, 1998). With the first description of NE81 in *Dictyostellium discoideum*, it has been suggested that the presence of lamins is connected to multicellularity, as *D. discoideum* has multicellular life stages (Batsios et al., 2012; Krüger et al., 2012). Later, lamin-like proteins have been identified in Choanoflagellata, Filasterea, Ichthyosporea, Dictyostelia, Rhizaria, Haptophyta, Dinoflagellata, Bicosoecida, Hyphochytridiomycota, Oomycota, and Ochrophyta (Kollmar, 2015; Koreny & Field, 2016).

The presence of lamins or lamin-like proteins in many lineages suggests a common origin, with an ancestor present in LECA (Kollmar, 2015; Koreny & Field, 2016). The ancestor protein was probably similar to lamin-B in Metazoa, but with additional heptad repeat interruption, closest to what is observed in oomycetes, dinoflagellates, and haptophytes (Koreny & Field, 2016). Early divergence can be the cause of the problematic identification of these proteins in protists (Kollmar, 2015; Koreny & Field, 2016). Lamins appear to have been lost in some lineages (e.g. yeast and Apicomplexa) (Hattier et al., 2007; Rout et al., 2017), in which cases, their function has been taken over by other proteins, in some cases by coiled-coil proteins with unclear relationship to lamins, such as NUP-1 of trypanosmatids (DuBois et al., 2012; Koreny & Field, 2016).

Lamins fall into type V of metazoan IFs and might also be ancestral to all other metazoan IFs. They are the main component of the nuclear lamina lying underneath the inner nuclear membrane. The role of lamins ranges from structural components of the nuclear lamina, and mechanical stabilization of the whole cell, to roles in chromatin organization, cell cycle, gene expression, and even DNA repair (Cohen et al., 2001; Dittmer & Misteli, 2011; Melcer & Gruenbaum, 2006).

The canonical protein architecture of lamin is tripartite, with head and tail domains flanking a central α-helix, which in turn consists of four segments with heptad periodicity and forms a coiled-coil. Several domains can be identified in their sequences: a nuclear localization signal, an immunoglobulin-like domain, a phosphorylation site for CDK1 (cyclin-dependent kinase 1), and a farnesylation site (the CaaX box) (Figure 10). Special significance should be given to the phosphorylation site, which mediates disassembly of the nuclear lamin (Peter et al., 1990; Ward & Kirschner, 1990), as phosphorylation sites are common in IF-like proteins, and could serve the same purpose as in lamins. Trends of loss of some of the domains, loss of complete genes, and duplication of genes have been observed both in metazoan IFs and in protist IF-like proteins (Kollmar, 2015). Duplications and subsequent differentiation may lead to a new role for the protist IF-like proteins, similar to the evolution of metazoan IF proteins (Koreny & Field, 2016).

Figure 10 – Schematic illustration of human lamin-A highlighting its conserved domains: coiled-coil rod domain (blue box), nuclear localization signal (red), immunoglobulin domain (yellow pentagon), CaaX box (orange). Phosphorylation site (not shown) is present in head domain. Number indicates length of protein in amino acid residues (Kollmar, 2015).

As mentioned above, of great importance in the study of lamins was the description of NE81 in *D. discoideum* (Krüger et al., 2012). NE81 shares with lamin its structure, localization, regulation throughout the cell cycle, and sites for posttranslational modification (Krüger et al., 2012). NE81 possesses the typical lamin tripartite structure, with head and tail domains connected by an α-helical coiled-coils-forming central rod. Its structure strongly resembles that of canonical lamins, missing only the lamins' Ig-like domain (Krüger et al., 2012). The canonical lamin phosphorylation and farnesylation sites have been conserved. Phosphorylation likely plays a role in the regulation of polymerization, preventing the formation of higher-order structures, such that the lamina collapses when phosphorylated by CDK1 (Batsios et al., 2012). NE81 localizes to the inner nuclear membrane and colocalizes with lamin B in mammalian cells (Krüger et al., 2012).

Association of NE81 with centrosomes has been reported, possibly mediating centrosomal linkage to the nucleus (Batsios et al., 2012). Knockout cells and cells with induced overexpression exhibit decreased nuclear as well as overall stability, and have multiple and/or misshaped nuclei (Krüger et al., 2012).

4.2 Alveolins

The presence of alveolins, also called inner membrane complex (IMC) proteins, is one of the unifying features of the infrakingdom Alveolata (Gould et al., 2008). As their name suggests, they are associated with alveoli and the IMC (Gubbels et al., 2004; Mann et al., 2002). Alveolins have been identified in all alveolate groups, ranging from two per species up to 50 in some apicomplexans, where they seem to be the most diverse (Gould et al., 2008). Their distribution pattern depends on the organization of the cytoskeleton. In ciliates, alveolins form a rectangular unit surrounding the cilia (Figure 11) (Gould et al., 2008). *Toxoplasma gondii* and other Apicomplexa integrate their alveolins between subpellicular microtubules on the cytoplasmic side of the alveoli and 10nm filaments – the subpellicular network, which runs through the whole cell body and forms a cup on the posterior end (Mann & Beckers, 2001). Individual proteins have different localizations in relation to other members of the family inside one organism (Gould et al., 2011).

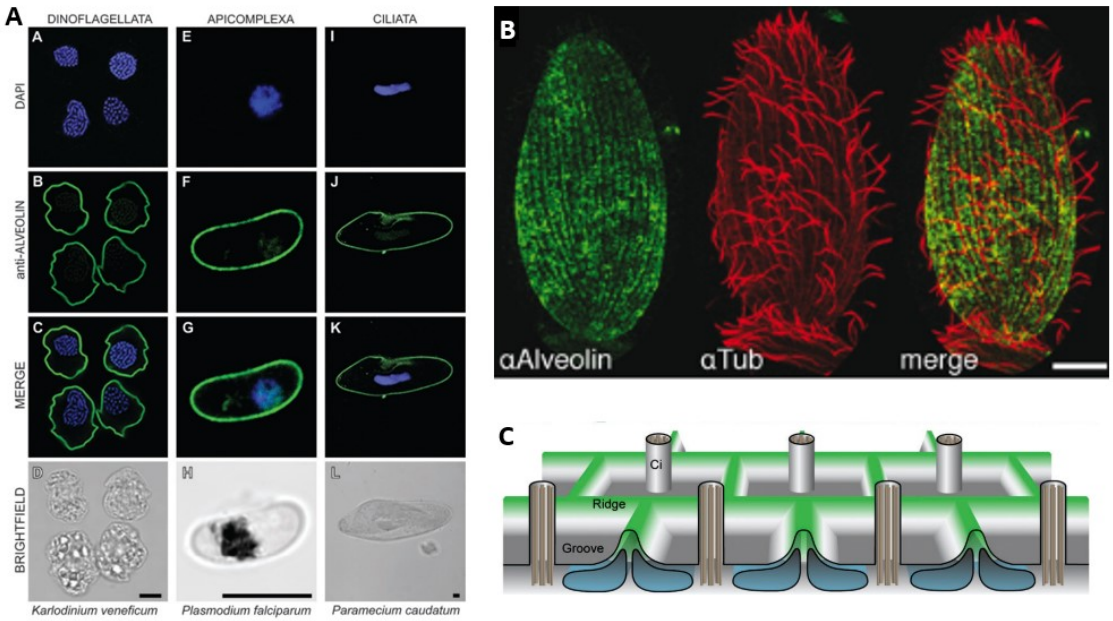


Figure 11- Localization of alveolins – A) comparison of different groups - dinoflagellates (*Karlodinium veneficum*), apicomplexans (*Plasmodium falciparum*) and ciliates (*Paramecium caudatum*) demonstrating presence of alveolins in cortical regions of cells (Gould et al., 2008); B) localization of alveolins in the ciliate *Tetrahymena thermophila* (El-Haddad et al., 2013); C) illustration of position of alveolins (in green) in *Paramecium caudatum*, showing ridges formed around cilia (Gould et al., 2008); all scale bars correspond to 10 µm.

Alveolins possess a repetitive rod region that is predicted to form coiled-coils (Gould et al., 2011; Mann & Beckers, 2001)., the size variability of which results in substantial differences in molecular mass. This rod region is flanked by unique terminal domains, where most of the variability between proteins is found (Gould et al., 2008). Repetitive sequences revolve around an EKIVEVP repeat, or subrepeats like EVVR or VPV, the repeats themselves range between 4 and 20 amino acids and sometimes can be generalized to pattern of negative, positive,

hydrophobic, hydrophobic, negative, hydrophobic, proline, and any residue (Gould et al., 2008). Repeats of *Plasmodium* sp. alveolins appear to be 12 residues long (Al-Khattaf et al., 2015). Assembly into oligomers before targeting has been proposed (Anderson-White et al., 2011). The repetitive sequences seem to be key for localization of alveolins to the cytoskeleton, with basal bodies likely acting as initial condensation centers. The N- and C-terminal domains participate in fine-tuned targeting of alveolins (El-Haddad et al., 2013).

The importance of alveolins for cell shape maintenance and mechanical stability has been observed, as knockout cells exhibit decreased resistance to pressure, loss of pellicle integrity, abnormal shape, or division impairment (El-Haddad et al., 2013; Khater et al., 2004; Mann et al., 2002; Mann & Beckers, 2001; Tremp et al., 2008). In *T. gondii*, alveolins play a role in the change of the rigidity of the subpellicular network from a rather fragile state, to a stable detergent-resistant one in the mature parasite. This is a consequence of proteolytic posttranslational modification at the C-terminus (Mann et al., 2002). Apicomplexan parasites, such as *Plasmodium* sp., seem to require alveolins for gliding and attachment, as this ability is impaired in knockout cells; their infectivity being compromised as a result (Khater et al., 2004; Mann & Beckers, 2001; Tremp et al., 2008; Tremp & Dessens, 2011; Volkmann et al., 2012). Some alveolins appear to be life-stage specific (Anderson-White et al., 2011; Khater et al., 2004; Tremp et al., 2008). Moreover, alveolins differ in their turnover, in that some are recycled in cell division (Hu et al., 2006) while others persist in maturation (Mann et al., 2002). Diversity of cell localization comes with expectations of slightly different functions for individual members of alveolins (Anderson-White et al., 2011; Tremp & Dessens, 2011).

Alveolins bear similarities to other IF-like proteins, such as plateins and articulins in both structure and presence of repetitive charged motifs (El-Haddad et al., 2013; Gould et al., 2008; Mann & Beckers, 2001).

4.3 Articulins

Articulins were first described as major components of the epiplasm of *Euglena gracilis* (Marrs & Bouck, 1992) and later identified in dinoflagellates and ciliates (Huttenlauch, 1995); however, no study of their precise localization exists. An 80-kDa articulin of *E. gracilis* interacts with the plasma membrane via a noncovalent bond to protein IP39 (Rosiere, 1990). In vitro, these proteins form a filamentous polymer with a diameter of about 15-20 nm, which forms higher-order structures, such as filaments, sheets, or tubes (Huttenlauch, 1995).

Articulin monomers consist of three domains – head, tail, and a central rod, which differ in amino acid composition (Huttenlauch, 1995; Marrs & Bouck, 1992). The rod domain consists of tandem repeats of 12 residues with a consensus sequence of VPVP--V-V-V-, which is unique for this family. If these repeats contain positively or negatively charged residues, their positions show an alternating pattern analogous to the V and P positions, where P positions are occupied by negatively charged residues, and the positively charged ones are usually found in V positions (Huttenlauch, 1995; Huttenlauch et al., 1998). In some cases, linkers (inserts 2-8 residues long) are present between the repeats (Huttenlauch, 1995). An alternative hypothesis states that the repeats might be only 6 residues long (Huttenlauch et al., 1998). The central rod domain is usually

predicted to form β-sheets hence separating the negatively and positively charged residues, but it is suggested it forms coiled-coils instead (Coffe et al., 1996).

In contrast to the proline- and valine-rich core, terminal domains show a different residue composition, which is more than 45% composed of glycine, alanine, and phenylalanine (Huttenlauch, 1995). Both domains also show a low abundance of charged residues (Huttenlauch et al., 1998). Head and tail domains may contain a phosphorylation site, which might be involved in assembly or disassembly of the filaments, as seen in other cytoskeletal proteins (Huttenlauch, 1995). Another pattern seen in non-core domains is a hydrophobic 7-amino acid repeat (with a consensus of APVTYGA), which might play a role in head-to-tail interaction between articulins (Marrs & Bouck, 1992).

4.4 Epiplasmins

The term epiplasmins has been used as a synonym for epiplasmic proteins. Due to the fact that the epiplasmic layer is composed of several different protein families, whose abundance is genus-specific, it is proposed using epiplasmins *sensu stricto* for a family of epiplasmic proteins found in *Paramecium* sp. and related proteins (Pomel et al., 2006). Therefore, Epc1 will be discussed separately.

Epiplasmins are found, as noted above, as components of the epiplasm in ciliates. Around 40 paralogs were identified in the *Paramecium tetraurelia* genome, while only two were found in *Tetrahymena thermophila*. Not only the number of paralogs but their relative contents in the epiplasm differ between species: for exaomple epiplasmins are the major component in *P. tetraurelia* and minor in *T. thermophila* (Pomel et al., 2006). One of the hallmark traits for IF-like proteins, which is also exhibited in epiplasmins, is their resistance to detergents like Triton-X and to salt extraction. Another hallmark is their ability to form higher-order structures from protofilaments *in vitro*, ranging from spheroids to curly ribbons to fibers up to 4 to 5 μm long and 70-150 nm thick (Coffe et al., 1996). No significant similarity to other IF-like proteins have been found; the closest matches were euglenoid articulins and ciliate plateins, with similarity scores lower than 35% (Pomel et al., 2006).

Epiplasmins can be situated in multiple areas in relation to the basal body unit (Figure 12): unit periphery (rim); over the whole unit with exception of the center (core); around the basal body (ring); on the basal body (basal) (Figure 12) (Aubusson-Fleury et al., 2013). They are also present in oral structures (Pomel et al., 2006). It is possible to separate epiplasmins based on their phylogeny into five groups, all of which were likely present in the common ancestor of both *Paramecium* sp. and *Tetrahymena* sp. (Aubusson-Fleury et al., 2013; Aury et al., 2006). Most paralogs are present in two copies, mirroring a recent whole-genome duplication; however, more ancient duplications can also be observed (Damaj et al., 2009). Proteins of one group share the same morphology; similarity in function is supported by identical phenotypes in case of depletion of epiplasmins of the same group within the cell. Related groups also show a correlation in their localization (Aubusson-Fleury et al., 2013).

Similar to articulins, epiplasmins take the form of a tripartite structure: a conserved central rod surrounded by two hydrophobic domains. We can also find repeats with consensus sequences

QPVQ-h and QxVV (where "h" stands for any hydrophilic residue), the ability to form coiled-coils has been attributed to these repeats (Coffe et al., 1996). Nevertheless, unlike in articulins these motifs do not form the entirety of the central domain, but merely flank the central sequence (Pomel et al., 2006). The repeated heptads were found to be missing in several proteins in this group, suggesting that they are not a good identifying marker for this family. Instead, it might be the middle part of the rod domain that seems to be remarkably conserved (Pomel et al., 2006); consensus motif [ERK]xx[VILT]EY[VIY] has been recognized in this part of the domain (Damaj et al., 2009). N- and C-terminal domains contain three structural motifs: repetitive PVQ rich motif; a hinge, which might also be a β-turn and a Y rich domain. Based on the presence of these motifs in N- and C-terminal sequences, the proteins can be classified as symmetric, asymmetric, or atypical (Damaj et al., 2009).

Division impairment resulting in abnormal phenotypes was observed in several cases: a) in cells expressing epiplasmins with GFP tags, probably due to overexpression (Aubusson-Fleury et al., 2013); b) during depletion by RNA interference (Damaj et al., 2009; Pomel et al., 2006); c) in the presence of a serine-threonine kinase inhibitor (Kaczanowska et al., 1996). Phosphorylation seems to be key for the elongation of epiplasmins with conserved phosphorylation sites (Damaj et al., 2009). It is likely, that epiplasmins contribute to the integrity of the ciliate cortex. It is also probable that they carry epigenetic information necessary for cell division (Damaj et al., 2009).

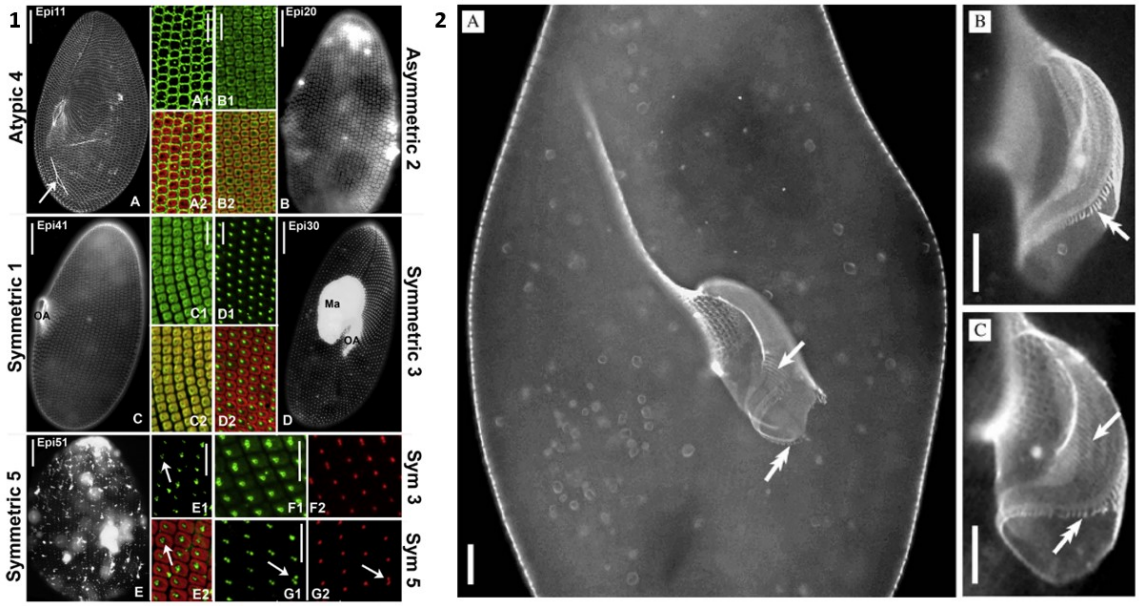


Figure 12 – Localization of epiplasmins in the cell – 1) localization of different epiplasmin groups (green): rim (Atypic 4), core (Asymmetric 2, Symmetric 1&3), ring (Symmetric 3) and basal body (Symmetric 5) –- scale bars: 20 µm (A-E) and 5 µm (A1-G1) (Aubusson-Fleury et al., 2013); 2) demonstration of presence of epiplasmins in oral apparatus of *Paramecium tetraurelia*, arrows represents cortical material of oral cavity, double arrow indicates the cytopharyngeal structures - scale bar 5 µm (Pomel et al., 2006).

4.5 EpiC

The most abundant component of the epiplasm of *Tetrahymena* sp. is called EpiC or Epc1 (Bouchard et al., 2001). This protein is built up from 40 aa long sequences repeated 25 times. These domains show similarity to lamins and cytoplasmic IFs of invertebrates.Ppoly-S motif on the C-terminus and a phosphorylation site csn be found in EpiC (Bouchard et al., 2001; Honts &

Williams, 2003). Assembly and crosslinking of these proteins have been observed *in vitro* (Honts & Williams, 2003). EpiC seems to take part in cell shape maintenance and cortical development. Knock out cells exhibit aberrant phenotypes such as overall roundness of cells, a presence of membranelles with clusters of cilia, and a misplacement of basal bodies. However,the most severe cortical defects were transient, its function was implying takenover by a different protein (Williams, 2004). Interestingly, EpiC migrates into the minus (knock-out) cell during conjugation (Figure 13) (Williams, 2004).

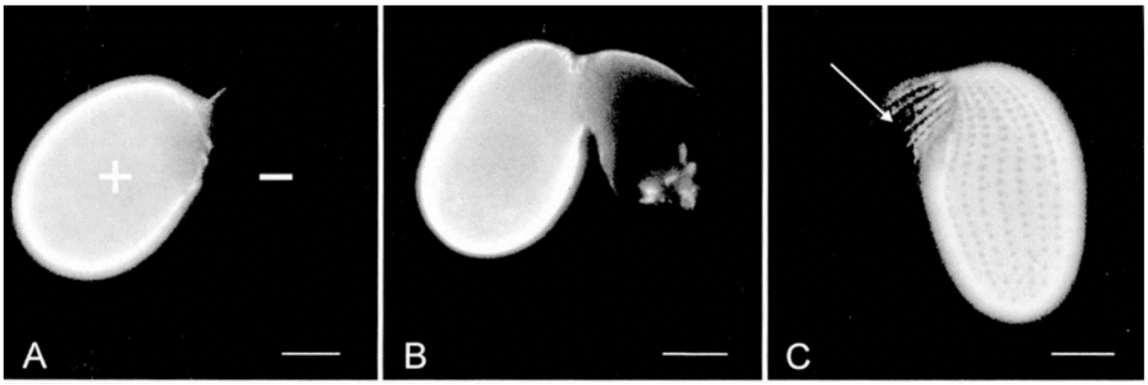


Figure 13 – transfer of EpiC from wild type *Tetrahymena* cell (+) into a knock-out cell (-) during conjugation, later this pattern extends from the anterior to the posterior end – scale bar 10 µm (Williams, 2004).

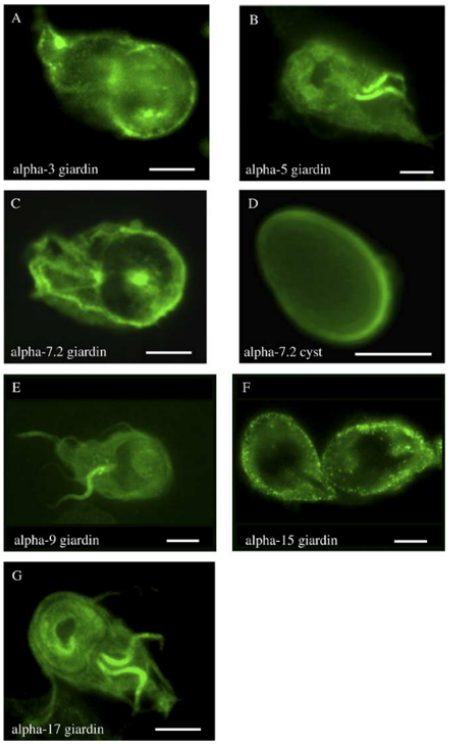
4.6 Giardins

Giardins were first identified as a component of microribbons in the ventral disk of *Giardia intestinalis* (Crossley & Holberton, 1983) and later distinguished by their patterns on two-dimensional electrophoresis. However, they do not correspond to a monophyletic protein family (Peattie et al., 1989). Their sizes range from 29 to 38 kDa (Peattie et al., 1989). Several groups are recognized: α-, β-, γ-, and δ-giardins (Peattie et al., 1989); each of them represents a very different group of proteins. While not all of them do fall into the category of IF-like proteins, a brief overview will be given about each of them. Differences in localization and therefore possibly also function of giardins have been observed in different assemblages of *G. intestinalis* (Feliziani et al., 2011), this might be related to modifications of the proteins (Palm et al., 2005).

4.6.1 Alpha giardins

Alpha-giardins are not IF-like proteins. This monophyletic family consists of 21 members and has been identified as a member of the annexin family based on their structure as well as on the ability to bind to the membrane in the presence of Ca^{2+} (Bauer et al., 1999; Morgan & Fernandéz, 1995; Weiland et al., 2005). Their convex-concave structure consists of four repeats of five α-helices each, which has been retained in evolution and is also found in plants, slime molds, nematodes, insects, vertebrates, and other protozoa (Gerke & Moss, 2002). They are capable of self-assembly, with probable mediation by phosphorylation (Vahrmann et al., 2008). The importance of α-giardins lies in their immunodominance, which is provided by a highly immunogenic epitope (aa 16-200 in α-1-giardin) in human infections by *G. intestinalis*, and which might be used as tools for diagnostic purposes (Palm et al., 2003; Weiland et al., 2003; Wenman et al., 1993). Alpha-giardins were localized to the plasma membrane or flagella, but are also found in the cytoskeletal fraction (Szkodowska et al., 2002; Vahrmann et al., 2008; Wei et al., 2010;

Weiland et al., 2005). It has been shown that *G. intestinalis* cells bind to glycosaminoglycans via some of these proteins, which might play a role in the attachment to the intestinal epithelium, mast cells, or connective tissue (Weiland et al., 2003). Other possible functions might be: stabilization of cytoskeleton by linking it to the cytoplasmic membrane; partaking in vesicle formation and membrane fusion (Szkodowska et al., 2002; Vahrmann et al., 2008; Weiland et al., 2005); stabilization of parasite-host attachment (Feliziani et al., 2011) or motility (Szkodowska et al., 2002). The immunogenic regions seem to be the least conserved part of the protein group (Alonso & Peattie, 1992). Expression levels of α-giardins are very high, nevertheless, overexpression leads to cell death or defective division and differentiation (Weiland et al., 2005).



Localisation of AU-1 epitope tagged alpha gardins

AU-1 tagged giardin protein	Main localisation
Alpha-1	Plasma membrane
Alpha-2	Plasma membrane, flagella
Alpha-3	Disc edge
Alpha-4	Lethal to the parasite
Alpha-5	Ventral flagella, diffuse disc
Alpha-6	Spotty cytoplasmatic
Alpha-7.1	Lethal to the parasite
Alpha-7.2	Plasma membrane, cyst wall associated
Alpha-7.3	Plasma membrane, cytoplasmatic
Alpha-8	Lethal to the parasite
Alpha-9	Flagella mainly ventral
Alpha-10	Ventral flagella
Alpha-11	Lethal to the parasite
Alpha-14	All flagella, weak median body
Alpha-15	Spotty cytoplasmatic close to pm
Alpha-16	Spotty cytoplasmatic close to pm
Alpha-17	Disc, flagella mainly ventral

Figure 14 – Localization of different GFP-tagged α giardins within the cell, with summary table of their localization – scale bar 5 µm (Weiland et al., 2005).

4.6.2 Beta giardins

Beta-giardins are SF-assemblin-like proteins; a group of IF-like proteins described below in greater detail. They seem to be abundant in the cell (Palm et al., 2005) and localize to the ventral disc specifically (Figure 15), but their precise position differs between assemblages (Feliziani et al., 2011). Heptad repeats with a skip residue give them the ability to form coiled coils, similar to IF proteins but unlike α-giardins (Alonso & Peattie, 1992; Holberton et al., 1988; Peattie, 1990). *In vitro*, they form 2.5 nm filaments that assemble into higher-order structures such as ribbons resembling the microribbons of *Giardia* sp. (Crossley & Holberton, 1985), and suggesting they might have a structural function (Feliziani et al., 2011).

4.6.3 Gamma giardins

Gamma-giardins are localized to the adhesive disk (Figure 15)(Nohria et al., 1992), more precisely to microribbons (Hagen et al., 2011; Palm et al., 2005). No similarity to α- or β-giardins

has been observed (Nohria et al., 1992). While an α-helix is predicted in the majority of the protein it is unlikely that this creates coiled coils, due to the absence of short repeats (Nohria et al., 1992).

4.6.4 Delta giardins

Delta-giardins (Elmendorf et al., 2001) present another presumed IF-like group (Hagen et al., 2011). They seem to be involved in the attachment of *G. intestinalis* to the intestinal epithelium (Jenkins et al., 2009).

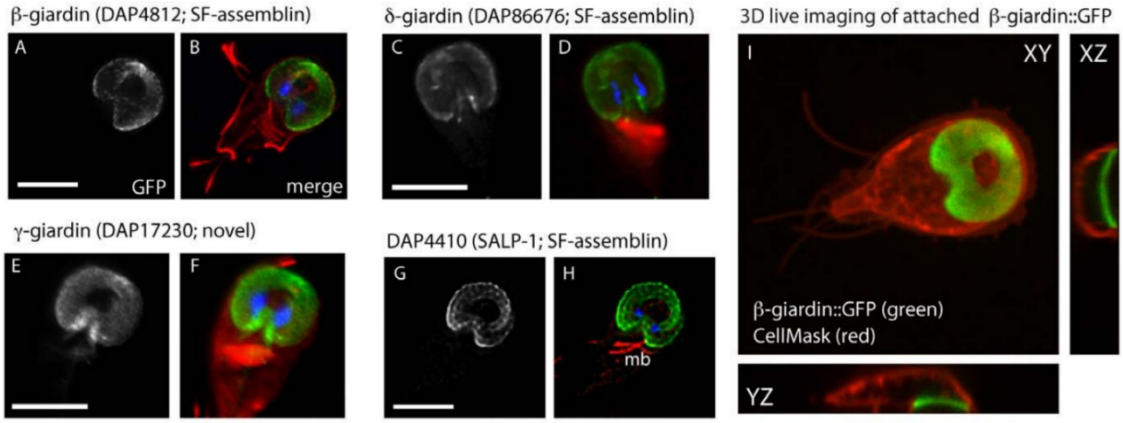


Figure 15 – Localization of GFP-tagged β-, γ- and δ-giardins (green) to the adhesive disk compared to adhesive disk marker (DAP4410) (red) – scale bar 5 μm (Hagen et al., 2011).

4.7 SF-assemblin

Striated fiber assemblin is the major component of striated microtubule associated fibers, also called SMAFs, system-I-fibers, SMACs, striated roots. SMAFs and similar structures are widespread but SF-assemblin has been studied the most in flagellated algae (*Spermatozopsis similis, Dunaliella bioculata, Chlamydomonas reinhardtii,* and *C. eugametos*) (Lechtreck & Melkonian, 1998). Similar fibers are observed for example in *Trichomonas* sp. and related species (Brugerolle & Viscogliosi, 1994; Viscogliosi & Brugerolle, 1994). Proteins with similarity to SF-assemblin were identified in Apicomplexa (Francia et al., 2012; Lechtreck, 2003) as well as Cilliata, Dinoflagelata, Bacillariophyceae, and Oomycota (Harper et al., 2009). As suggested by their name, they are associated with flagellar root microtubules (Patel et al., 1992) for about 75% of their length (Lechtreck et al., 1996). The association seems to be in part thanks to the affinity of SF-assemblin to acetylated tubulin (Lechtreck et al., 2002). Interphase cells show the presence of these proteins in association with all four microtubular roots. In metaphase, two dot-like structures are seen near the spindle poles, which associate together briefly in anaphase and subsequently give rise to new fibers in telophase (Lechtreck & Silflow, 1997).

SF-assemblin forms polarized fibers with distinct minus (hooked or blunt) and plus (tapered) ends, unlike other IF and IF-like fibers, which are usually not polarized. The cross-section of the fiber is either circular or oval (Patel et al., 1992). The length of SMAFs is between 100 and 1400 nm, and their width tapers from 55-60 nm to 10-20 nm (Patel et al., 1992). *In vitro*, SF-assemblin assembles into very similar structures (Patel et al., 1992). The filaments are formed from 2 nm protofilaments (Lechtreck et al., 1996; Weber et al., 1993) of individual proteins organized in parallel (Patel et al., 1992). In electron microscopy, these fibers exhibit a repetitive pattern

approximately 28 nm long, and this phenomenon is likely explained by superposition of protofilaments (Weber et al., 1993).

SF-assemblin is built differently from the tripartite structure seen in other IF proteins, by featuring only one prominent nonhelical domain on its N-terminus and alpha-helical segmented coiled-coil-forming rod taking up the rest of the molecule, with a cluster of acidic residues on the C-terminus (Weber et al., 1993). The approximately 30-aa long N-terminal domain is essential for the assembly of SMAFs (Lechtreck, 1998). The head domain features several potential phosphorylation sites, likely involved in the dynamics of the protein (Weber et al., 1993). The repeat pattern of the coiled-coil domain is 29 residues long, specifically 28 (4 heptads) and one skip residue, for which glutamine or glutamic acid are preferred (Weber et al., 1993). The removal of one or two of these skip residues appears to have no effect on further assembly unlike their substitution with a different residue (e.g. alanine) (Lechtreck, 1998). Interestingly, cysteines with the potential to form disulfide bonds between dimers are present in the α-helical domains (Lechtreck & Melkonian, 1998; Weber et al., 1993).

It is suggested that SF-assemblin might take part in the reestablishment of the microtubular root system after cell division, stabilizing the microtubules (Lechtreck & Silflow, 1997). Other studies suggest that SF-assemblin might function as a nucleation center (Lechtreck et al., 2002). In the case of underexpression of SF-assemblin, cells exhibit abnormal phenotypes such as a reduced number of flagella (Lechtreck et al., 2002).

Beta-giardins show a similarity to SF-assemblin in structure, 29 residue repeats, and similar preferences for chemical properties of used residues, nevertheless their sequence identity is low (Holberton et al., 1988; Weber et al., 1993). Skip residues seem to be conserved in all observed proteins (Lechtreck & Silflow, 1997). The presence of such similar proteins in very distinct organisms might indicate their more abundant presence in eukaryotes (Lechtreck et al., 1996; Lechtreck & Melkonian, 1998). A low sequence identity of only 57% between individual SF-assemblins was demonstrated between Chlorophyceae algae *Spermatozopsis similis* and *Dunaliella bioculata*, (Lechtreck et al., 1996).

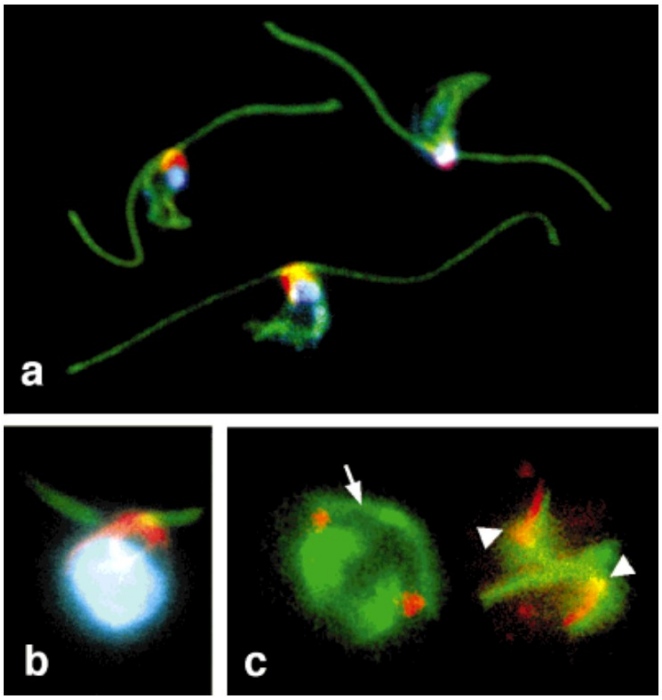


Figure 16 - Localization of SF-assemblin (red) in *Spermatozopsis similis* (a), *Dunaliella bioculata* (b) and *Chlamydomonas reinhardtii*, additionally tagged with DAPI and tubulin (green) (Lechtreck & Melkonian, 1998).

4.8 Plateins

This family of proteins has been identified in euplotid ciliates, and, based on two-dimensional immunoblots, sorted into two groups: α and β/γ (Kloetzel, 1991). Plateins are ubiquely contained inside of the alveoli (Figure 17) (Kloetzel, 1991; Kloetzel et al., 2003b; Williams et al., 1989), which gives them interesting properties dissimilar to other IF-like proteins (Kloetzel et al., 2003a). While their main repeats are similar to the VPV coiled-coil-forming motif of articulins, platiens contain more acidic residues (Kloetzel et al., 2003a). Acidic residues might play a role in interaction with Ca^{2+} present in alveoli (Kloetzel et al., 2003a). The consensus of the dodecameric repeats differs between α- and β/γ-plateins and corresponds to VP(R/H)T(Y/V)EY(Q/V)EZ(I/V)T and V(P/D)E(V/P)EY(R/V)TRYZ(T/V), respectively. As expected from their position in the cell, they possess an N-terminal start-transfer signal sequence targeting them to alveoli, which might be cleaved in the process of maturation (Kloetzel et al., 2003a). Surprisingly, many residues seem to be available for phosphorylation. This property is more prominent in the β/γ plateins, once again suggesting a possible means of regulation (Kloetzel et al., 2003b). Formation of the platein polymers may have a role in assembling new alveoli during cell division (Kloetzel, 1991) as well as in the maturation of alveoli, which demonstrates as increased resistance to detergents of the mature alveoli (Kloetzel et al., 2003b).

Plateins bear a striking similarity to articulins, not only in the presence of analogs of VPV repeats, but also by possession of pentameric repeats initiated by a proline residue (Kloetzel et al., 2003a). Nevertheless, they are built a little differently, because their rod domain is rather closer to the C-terminus, and some plateins nearly lack the C-terminal domain altogether (Kloetzel et al., 2003b). Other differences lie in the localization: plateins are found inside the

alveoli and articulins in the epiplasm. It is proposed that α-plateins originated from articulins and later gave origin to β/γ-plateins by gene duplication (Kloetzel et al., 2003a).

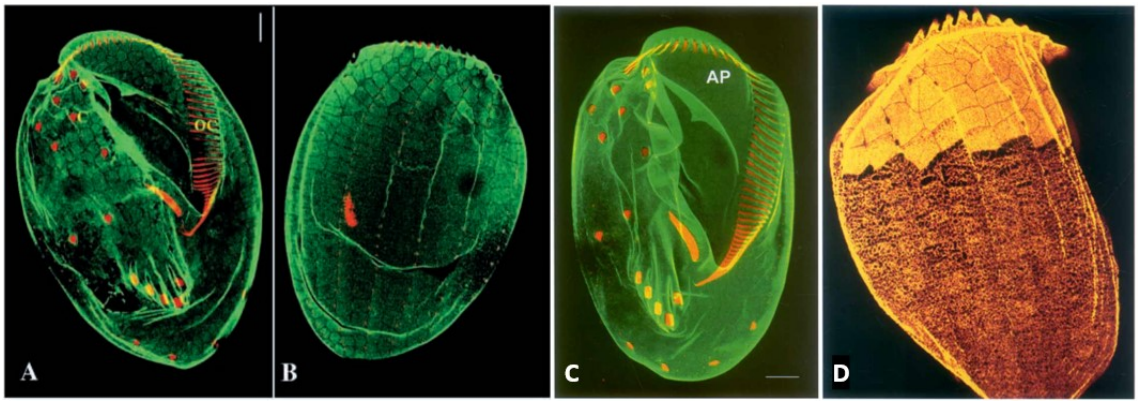


Figure 17 – Localization of plateins in *Euplotes* sp. – A-C plateins in green, centrin in red (note visible plates in A-C); D) shows turnover of plates after division, with old plates on top (bright yellow) and new on bottom part of the cell - scale bars 10 μm (Kloetzel et al., 2003a, 2003b).

4.9 Tetrins

Tetrins are found mainly in the oral filaments of *Tetrahymena* sp. (Dress et al., 1992; Honts & Williams, 1990; McLaughlin & Buhse, 2004), but are also present in the coarse filamentous reticulum (CFR) and undulating membrane filaments (Figure 18) (McLaughlin & Buhse, 2004); a role in the stabilization of these structures is proposed (Clerot et al., 2001). Association with microtubules, specifically basal bodies, was found, although not always a particularly close one (Dress et al., 1992). Several variants of tetrins are recognized, which differ greatly in their sequence, but share common structures and localizations (Brimmer & Weber, 2000; Dress et al., 1992). They take up the classical tripartite arrangement, with an over-600-aa-long α-helical central rod domain, while heptad repeats are present, it is more likely that they form segmented instead of continuous coiled-coils (Brimmer & Weber, 2000; Honts & Williams, 1990). Formation of 3-5-nm filaments and subsequent assembly into thicker filament bundles was detected both *in vivo* and *in vitro* (Honts & Williams, 1990). Their disassembly upon phosphorylation preceding cytokinesis is proposed (Honts & Williams, 2003).

Some cross-reaction has been shown between anti-tetrin antibodies and *P. tetraurelia* filament-forming proteins, implying that related proteins are widespread, although further research is needed for confirmation (Clerot et al., 2001). Relations to other IF-like proteins are unclear (Brimmer & Weber, 2000; Honts & Williams, 2003).

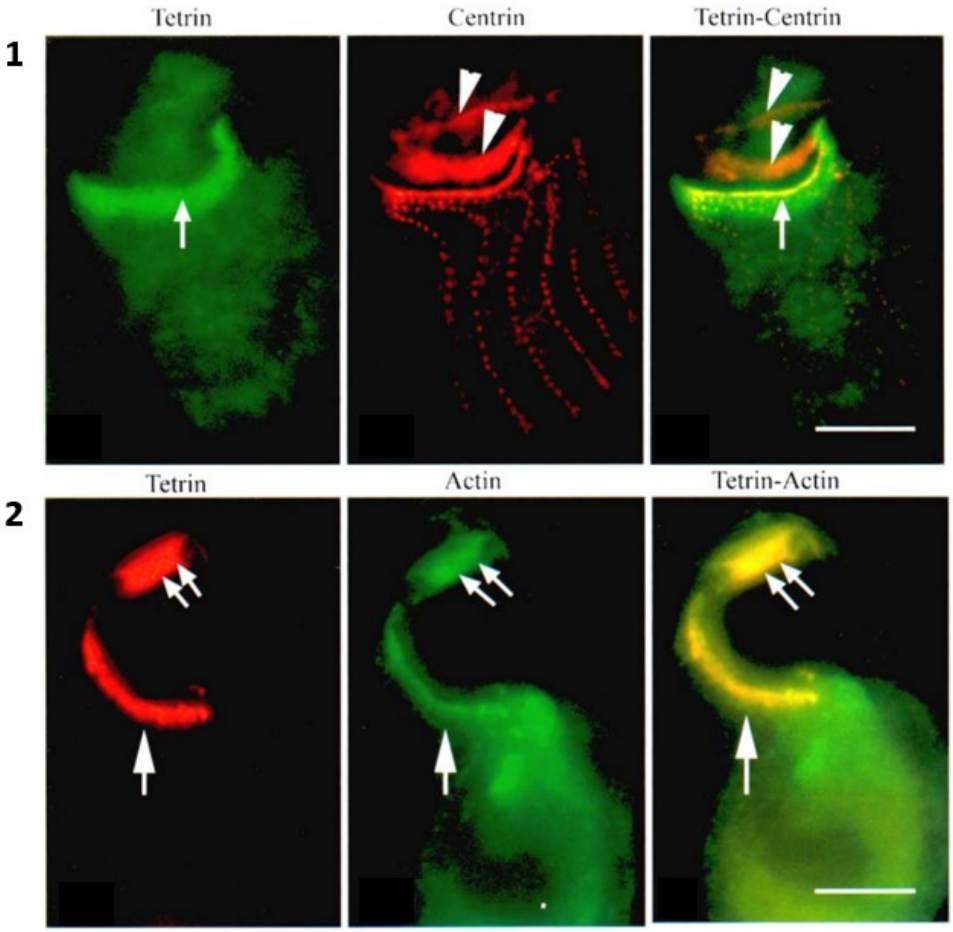


Figure 18 - Localization of tetrin in *Tetrahymena* – 1) shows localization to coarse filamentous reticulum (arrow) and fine filamentous reticulum (arrowheads); 2) shows localization to coarse filamentous reticulum and cross-connectives (pair of arrows)- scale bars 25 μm (McLaughlin & Buhse, 2004).

4.10 Other protein families

The following part is dedicated to less studied proteins with features similar to IF proteins with filament-forming abilities, possessing repetitive motifs, and predicted to form long coiled-coils (Preisner et al., 2018). Nevertheless, their recognition as IF-like proteins is not always clear.

4.10.1 Head-stalk proteins

This group of proteins has been identified in the genome of *Giardia intestinalis*, based on their structure. In total, over 30 head-stalk proteins have been identified in the *G. intestinalis* genome. They share the presence of several ankyrin repeats and a long coiled-coil rod domain, which is an uncommon combination. The 33-aa-long ankyrin repeats create a helix-turn-helix structure and likely mediate protein-protein interactions, these domains can be found on one end of the molecule or flank the central rod. The consensus sequence for one ankyrin repeats in one of the identified head-stalk proteins is -G-TALM-AAE-G-TD-V-L-YE-. This α-helical rod has a high prediction for the formation of coiled-coils and contains many interruptions, ranging from one to six amino acids in length. An Ability to bind GTP or ATP has been proposed. Interaction of these proteins with the cytoskeleton has been proposed (Elmendorf et al., 2005). One of the described head-stalk proteins localizes to the intracellular portions of axonemes of the two anterior flagella, giving the illusion of "eyebrows" (Figure 19) (Elmendorf et al., 2005). Another

study described a protein localizing to the plasma membrane in trophozoites and cytoplasm of cysts (Bae et al., 2009).

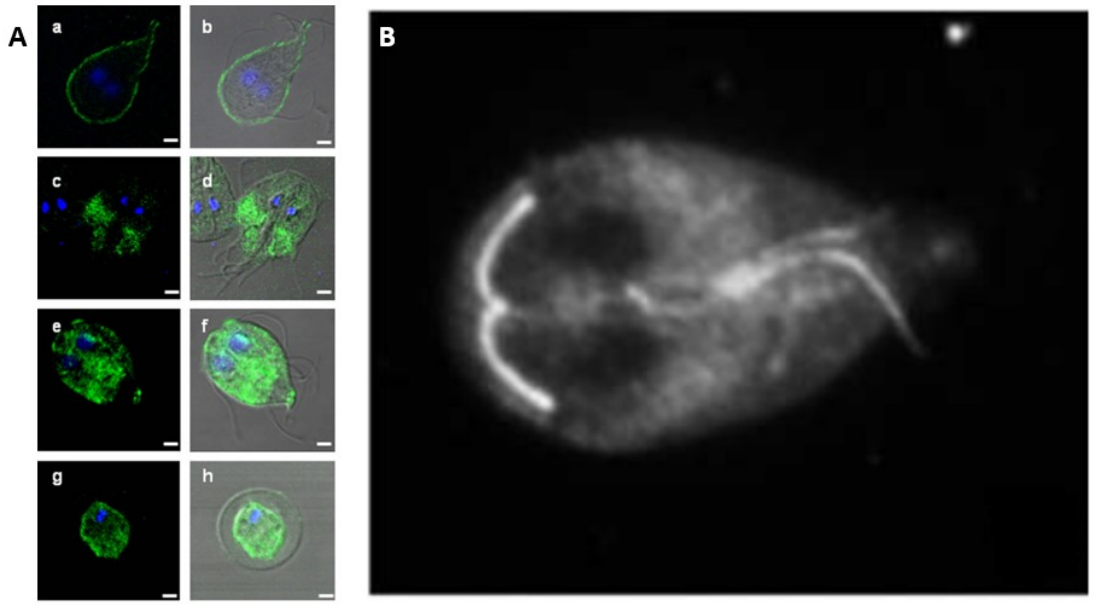


Figure 19 – Localization of head-stalk proteins in *Giardia intestinalis* – A) shows shift in localization of head-stalk proteins (green) in trophozoite during encystation: trophozoite cell (a) at 12 h (c), 24 h (e), and 48 h post-induction of encystation (g), – scale bar 2 µm (Bae et al., 2009) B) "eyebrow" localization (Elmendorf et al., 2005).

4.10.2 Septins

Septins are a group of GTP-binding proteins with filament-forming ability. They have been mainly characterized in opisthokonts. It has been shown septins are capable of filament formtaion *in vitro* (Nishihama et al., 2011). Their characterization is based on the presence of several domains: CDC GTP-binding domain with three motifs (G1, G3, G4), a polybasic region, a septin unique element, a C-terminal coiled-coil, conserved single residues in Sep1-4 (Figure 20). The function of the conserved single residues is unclear. The GTPase domain functions in binding and later hydrolysis of GTP. Polybasic regions interact with phospholipids. Coiled-coil domains are not present in all septins, but are common in opisthokonts and has been identified in two protist proteins (Nishihama et al., 2011; Onishi & Pringle, 2016). The presumed function of these domains is the mediation of interaction between individual septins. The septin-unique domain might also participate in filament formation. Studies in both animals and fungi point to a plethora of functions: cell and nuclear division, formation of barriers between compartments, vesicle trafficking, apoptosis, and cytoskeletal organization (Momany et al., 2008). The relationship with other IF and IF-like proteins is unclear.

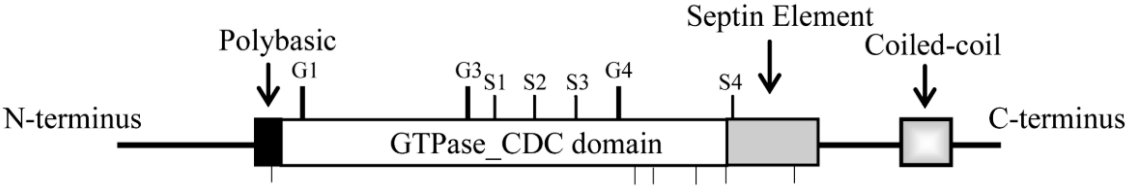


Figure 20 – Typical domain structure of septins: polybasic region, GTP CDC binding domain, septin unique element, a coiled-coil domain, conserved motifs G1-4, and conserved residues S1-4 (Momany et al., 2008).

Later studies showed the presence of septins in many protist lineages: chlorophyte algae, brown algae, Cryptophyta, Haptophyta, Rhizaria, Ciliata, and diatoms (Nishihama et al., 2011; Włoga et al., 2008; Yamazaki et al., 2013). Septins are not found in Amoebozoa, most plant lineages (except for chlorophytes and brown algae), and Excavata (Onishi & Pringle, 2016). No septin homolog was found in *Naegleria gruberi*, *N. fowleri*, *Trypanosoma brucei*, *T. cruzi*, *Leishmania major*, and *Trichomonas vaginalis*. One exception is a one putative septin homolog in *Giardia intestinalis* (Onishi & Pringle, 2016). The absence of septins in land plants might be due to their different cell division strategy involving cell plates, resulting in loss of the gene (Yamazaki et al., 2013). The absence in Excavata is more peculiar and suggests two possible explanations; ancestral septin was present in LECA but was lost in Excavata shortly after their divergence from the rest of the eukaryotes, or septins evolved after the divergence of excavates (Onishi & Pringle, 2016). Septin-like sequences lacking some of the hallmark septin domains have been reported from *G. intestinalis*, *Entamoeba histolytica,* and *Symbiodinium minutum*, this suggests they belong to a wider group of related proteins, which might have common ancestry with septins but are not *bona fide* septins (Nishihama et al., 2011; Onishi & Pringle, 2016).

The function of protist septins is unknown (Onishi & Pringle, 2016). A filament ring composed of septins forms at the division site of flagellated algae undergoing cytokinesis, suggesting they might have a role in cell division (Yamazaki et al., 2013). Studies on ciliates have shown a possible role in mitochondrial organization, autophagy, and organization of the endoplasmic reticulum (Włoga et al., 2008). It has also been hypothesized that septins are involved in the organization of cilia (Nishihama et al., 2011).

4.10.3 Apical polar ring proteins

The apical polar ring (APR) is a structure unique to apicomplexans, where it serves as a MTOC. However, its molecular composition is not well described. This text will focus on two proteins identified within the structure: RNG1 and RNG2.

RNG1 was identified in *T. gondii*, with homologs identified in *Neospora caninum* and *Sarcocystis neurona*. There, it forms a component of the APR integrating at the end of cell division. Difficulties with the description of related proteins tie in with its low complexity. The characteristic that would help identify a related group of proteins might be the abundance of proline (up to 25%). Knock-outs of this gene were unsuccessful in pointing to its essentiality for the parasite (Tran et al., 2010).

RNG2 was first identified as charged repeat motive protein in *T. gondii*, therefore has a high likelihood of forming coiled-coils (Gould et al., 2011). A cone connecting the APR and conoid is formed by this protein, which starts to assemble early during mitosis. The N-terminus remains connected to the conoid while the C-terminus interacts with the APR (Figure 21). RNG2 seems to have a role in the regulation of microneme secretion and therefore affects invasion abilities and motility of the parasites. Homolog of RNG1 with low sequence identity was identified in *Neospora caninum*; and it is proposed other Apicomplexa might bear similar protein (Katris et al., 2014).

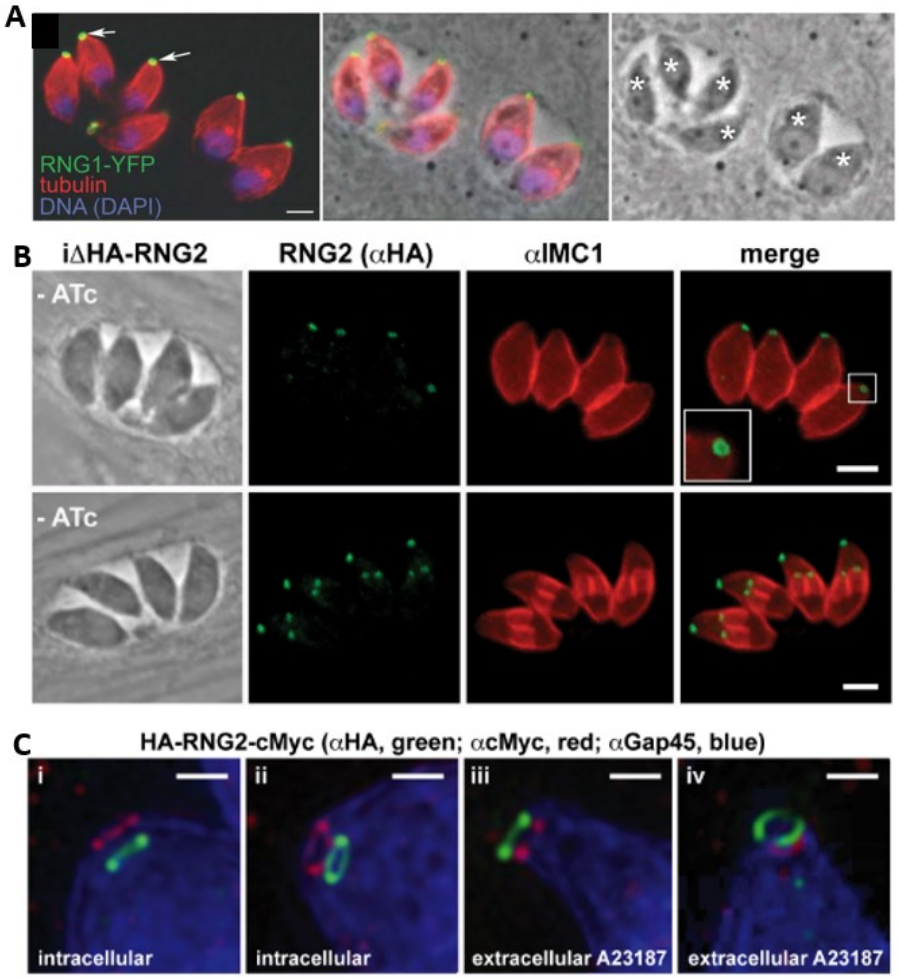


Figure 21 – localization of APR proteins in tachyzoites of *T. gondii* – A) localization of RNG1 to APR (Tran et al., 2010); B) localization of RNG2 (green) in tachyzoites and in dividing cells, red represents marker of cell pellicle IMC1 - scale bar 3 µm; C) demonstration of different localization of the two ends of RNG2 (green for conoid bound N-terminus, red for APC bound C-terminus, blue for GAP45), and their reversion during conoid extrusion (III-IV) – scale bar 0.5 µm (Katris et al., 2014).

5 IF-like proteins in cytoskeletons of protists

Protist cells vary in shape immensely. While we have a pretty good notion about their morphology, the protein composition of their cytoskeleton, aside from actin, tubulin, and proteins associated with them, remains largely unknown. Cytoskeletons of protists often feature striated filaments of mostly uncharacterized protein composition. Studies of certain striated fibers identified IF-like proteins as their main components. This might hint at a more ubiquitous presence of IFs in these thus-far unstudied filaments. Selected unique structures of protist models are presented below with the current knowledge on their protein composition.

5.1 Striated fibers of *Chlamydomonas*

It seems appropriate to mention *Chlamydomonas reinhardtii* first, as its striated fiber gave itsname to SF-assemblin, a predominant component of the striated fiber underlying the microtubular roots (marked in orange in Figure 22). This protein appears to have homologs in an increasing number of organisms, including Apicomplexa (Francia et al., 2012; Lechtreck, 2003), Cilliata, Dinoflagelata, Bacilariophyceae and Oomycota (Harper et al., 2009). Fibers adjacent to the basal bodies of *C. reinhardtii*, like distal striated fiber andthe nucleo-basa-body connector are formed by centrin (marked in red in Figure 22) (Dutcher & O'Toole, 2016). An additional coiled-coil protein DIP13 (deflagellation inducible protein), was found to be a prominent component of the flagellar basal apparatus. DIP13 is likely needed for proper cell division, and its homologs have been found in *G. intestinalis*, *L. major*, *T. brucei*, *P. falciparum*, *Cryptosporidium parvum* and mammals (Pfannenschmid et al., 2003; Schoppmeier et al., 2005). Nevertheless, the compositions of some of *C. reinhardtii* striated fibers such as. proximal fibers (marked in violet in Figure 22), are still unknown (Dutcher & O'Toole, 2016). *C. reinhardtii* is also reported to possess members of all known tubulin families (Dutcher et al., 2002; Dutcher & Trabuco, 1998; Findeisen et al., 2014).

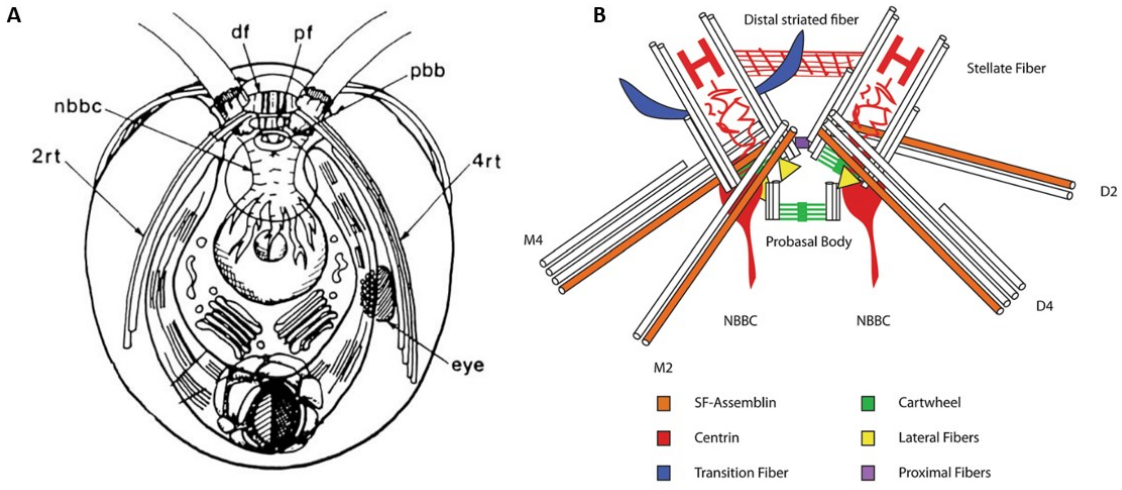


Figure 22 – Morphology of *C. reinhardtii* – A) cytoskeletal elements around basal body complex: df - distal fiber, pf - proximal fiber, nbbc - nucleobasal body connectors, 2rt - two-membered rootlets, 4rt - four-membered rootlets, pbb - basal bodies (Dutcher, 1995); B) detail of basal body, note SF-assemblin fibers in orange: NBBC - nucleo-basal body connector, M4 and D4 - four-membered rootlet microtubules, M2 and D2 - two-membered rootlet microtubules - (Dutcher & O'Toole, 2016).

5.2 Adhesive disc of *Giardia*

The most intriguing cytoskeletal structure of *Giardia* is undoubtedly the ventral disc used for attachment to the host intestinal epithelium. The ventral disc includes three main modules: a spiral array of microtubules, tri-laminar microribbons that are attached at right angles to the microtubules, and finally the crossbridges that link the microribbons (Figure 23) (Dawson, 2010). Proteomic studies revealed several major components of the disk, along with α- and β-tubulin, members of the α-giardin annexin family, SF-assemblin-like proteins, including SALP-1, β-, γ-, and δ-giardins, a homolog of Mp1p (a protein involved in adhesion in yeast), head-stalk proteins and numerous hypothetical proteins (Elmendorf et al., 2005; Hagen et al., 2011; Lourenço et al., 2012; Palm et al., 2005; Schwartz et al., 2012). The SF-assemblin-like proteins are confirmed to be components of the microribbons (Hagen et al., 2011). Other interesting components are the head-stalk proteins bearing the uncommon combination of long coiled coils and ankyrin repeats (Elmendorf et al., 2005). *G. intestinalis* is also the only known excavate to possess a putative septin homolog (Onishi & Pringle, 2016).

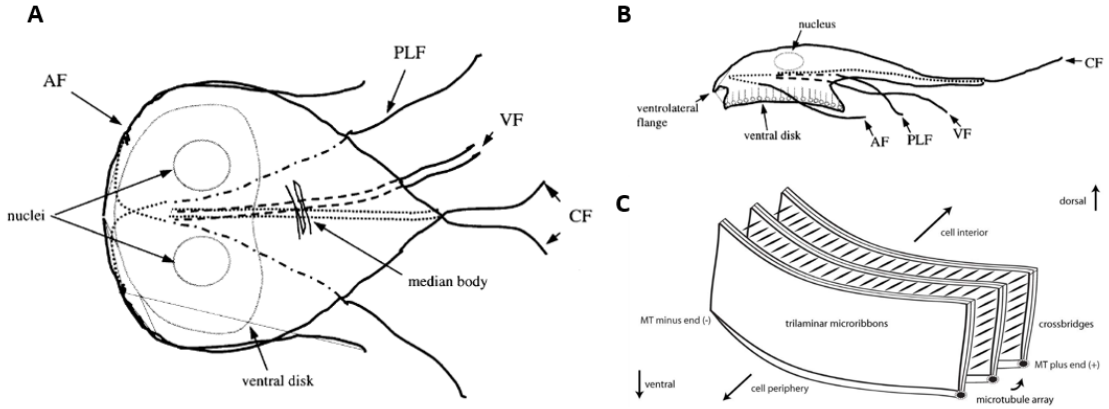


Figure 23 – Morphology of *G. intestinalis* trophozoite cytoskeleton – A) dorsal perspective; B) lateral perspective – AF - anterior flagella; CF - caudal flagella; PLF - posterior lateral flagella; VF - ventral flagella (Elmendorf et al., 2003), C) cross-section of ventral disc showing microribbons connected by crossbridges (Hagen et al., 2011).

5.3 Conoid and subpellicular network of Apicomplexa

The trademark component of the apicomplexan cytoskeleton is the conoid (Figure 24). The base structure of the conoid is microtubular in nature, although this is arranged into unique ribbon polymers (Hu et al., 2002). Over 250 proteins have been identified in the conoid, among them RNG1, RNG2, kinesin A, and a family of alveolins - the IMC proteins (Gómez de León et al., 2014; Long et al., 2017). Interesting is the presence of the ankyrin repeat protein CPH, a protein well conserved in Apicomplexa. The importance of CPH lies in the stabilization of the conoid and the integration of many other proteins into the structure (Long et al., 2017). *Toxoplasma gondii* also possesses homologs of SF-assemblins SFA2 and SFA3. These form a fiber that links centrosomes to the tips of forming daughter cells, and as such are required for the assembly of daughter cells (Francia et al., 2012). Well-studied components of cortical alveoli (Figure 24) are IF-like hallmark proteins of Alveolata, the alveolins (Gould et al., 2008). In *T. gondii* cells, alveolins integrate into the subpellicular network and play a role in stability and maturation of the network (Mann et al., 2002; Mann & Beckers, 2001), while *Plasmodium* alveolins are involved

in the attachment and gliding of the cell (Khater et al., 2004; Tremp et al., 2008; Tremp & Dessens, 2011). Also of interest is the fact that apicomplexans form shorter actin filaments as compared to other organisms (Schmitz et al., 2005; Schüler et al., 2005a, 2005b; Schüler & Matuschewski, 2006).

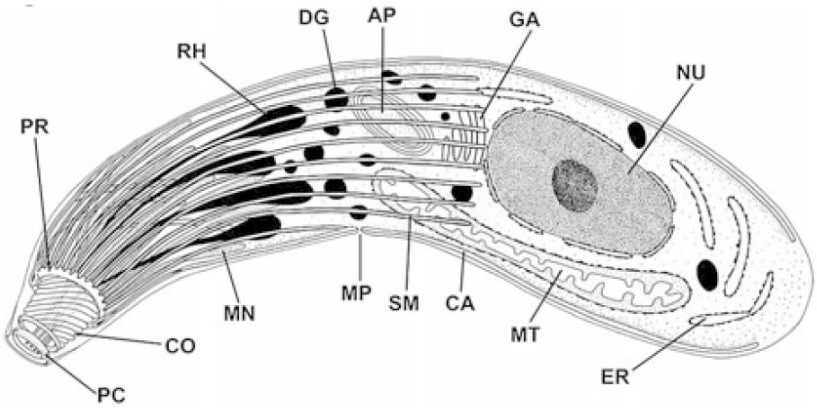


Figure 24 – Illustration of zoite of Apicomplexa – AP - apicoplast, CA -cortical alveoli, CO - conoid, DG - dense granules, ER - endoplasmic reticulum, GA - Golgi apparatus, MN - micronemes, MP - microporus, MT - mitochondrion, NU - nucleus, PC - pre-conoidal rings, PR - polar ring, RH - rhoptries, SM - subpellicular microtubules (Votýpka et al., 2017).

5.4 Pellicle and paraflagellar rod of Euglenoza

Two main components of the cytoskeleton of euglenozoans immediately attract attention, the paraxonemal or paraflagellar rod (PFR) and the pellicle (Figure 25). The pellicle includes the epiplasm, a structure composed of strips that underlies the plasma membrane. The main components of these epiplasmic strips are the IF-like proteins articulins (yellow in Figure 25) (Leander et al., 2007; Marrs & Bouck, 1992). The strips interlock by their heel and toe segments, in a so-called articulation zone. The whole structure is underlain by microtubules and the endoplasmic reticulum (red and pink in Figure 25, respectively) (Cavalier-Smith, 2017; Leander et al., 2007). These strips are the basis for euglenoid movement, a process that is still not entirely understood but likely is not dependent on either myosins or dyneins. It is proposed that this movement is caused by the contraction of centrin fibers stimulated by Ca^{2+} (Cavalier-Smith, 2017).

The PFR rod is a filamentous structure running alongside the axoneme of flagella of all Euglenozoa species, and so it is a prominent component of the trypanosomatid cytoskeleton (Figure 25). The PFR is a complex structure composed of a plethora of proteins, among them myosin, γ-tubulin, calmodulin, and adenylate kinases (Portman & Gull, 2010; Subota et al., 2014). The main two protein components of the lattice are called the PFR1 and PFR2, these related proteins possess a calmodulin-binding domain. They seem to originate from a single ancestor that underwent a duplication prior to the divergence of Euglenida and Kinetoplastida (Portman & Gull, 2010; Talke & Preisfeld, 2002). Despite sharing some properties with IF-like proteins (amino acid composition, degree of α-helicity, and solubility), they do not belong in this group as no short repetitive sequences enabling the formation of coiled-coils were found (Schlaeppi et al., 1989).

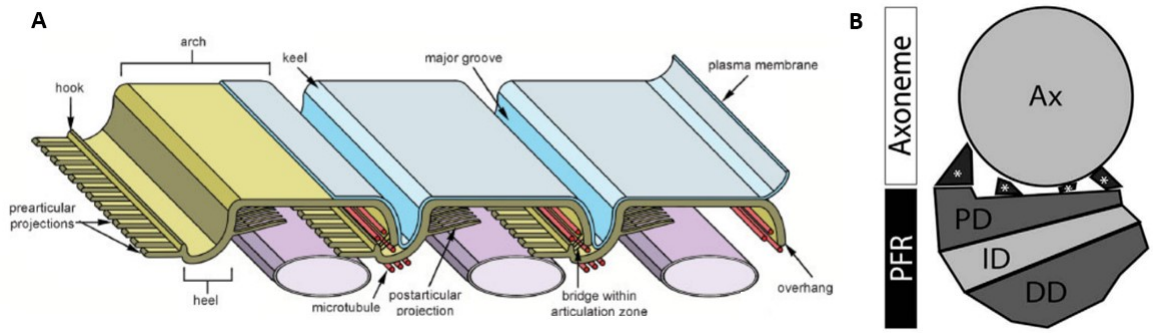


Figure 25 – A) illustration of articulin strip (yellow) in pellicle of euglenids (Leander et al., 2007); B) illustration of paraxonemal rod (PFR) of *Trypanosoma brucei* flagellum – PD - proximal domain; ID - intermediate domain; DD - distal domain (Portman & Gull, 2010).

5.5 Striated fibers of trichomonads

While the axostyle and pelta, prominent cytoskeletal structures of trichomonads, are known to consist primarily of tubulin, the molecular basis of other components is less clear. These include the costa, a prominent striated structure supporting the undulating membrane, and several other striated fibers, for example, the parabasal fibers (Figure 26), filaments with structural similarity to the costa that offer support to the Golgi apparatus (Benchimol, 2004; Čepička et al., 2017). Several putative IF-like proteins were localized in *T. vaginalis* to the costa, as well as to the axostyle, the pelta, in proximity to the nucleus, and possibly even to the parabasal filaments (Figure 26) (Preisner et al., 2016). Over 100 distinct proteins were identified in the isolated costa fraction, including actin and tubulin (de Andrade Rosa et al., 2017).

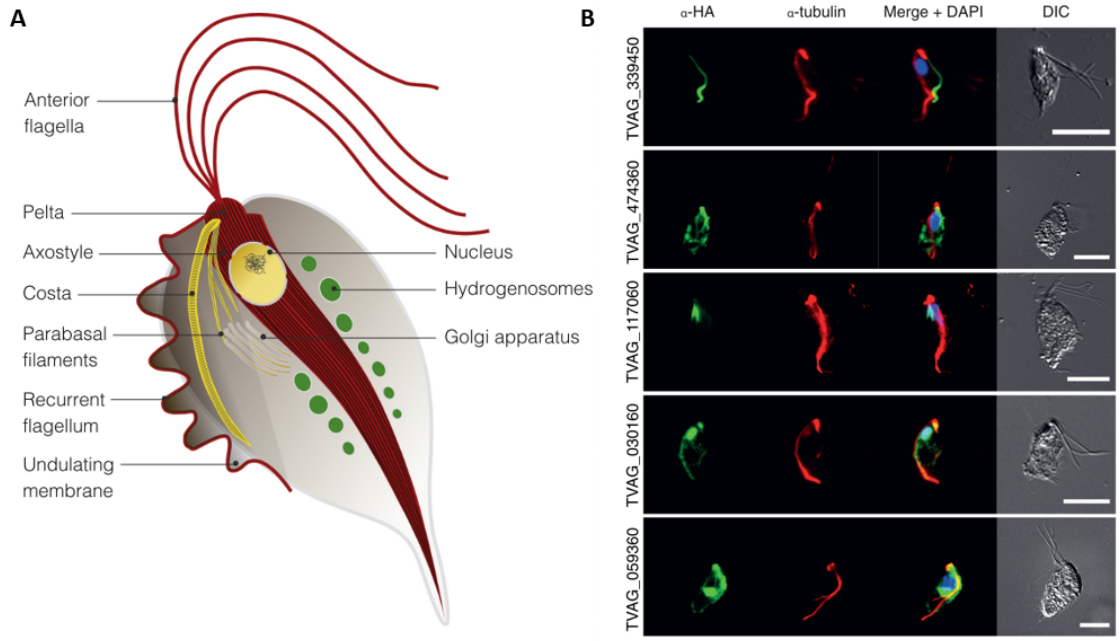


Figure 26 – A) Illustration of cytoskeleton of *T. vaginalis*; B) localization of putative IF-like proteins in *T. vaginalis* (green) with labeled α-tubulin (red) and DAPI (blue): TVAG_339450 – likely a major component of the costa, TVAG_474360 – localizes in proximity to nucleus, TVAG_117060 – putative component of t parabasal filaments, TVAG_030160 – colocalizes with pelta, axostyle and near tnucleus, TVAG_059360 - forms net around the nucleus that expands to rest of cell, scale bar 10 µm (Preisner et al., 2016).

6 Conclusion

The three main cytoskeletal domains of eukaryotic cells differ from one another and possess characteristics that make them unique both on the level of individual proteins and on the level of the filaments resulting from their polymerization. Actin and tubulin are members of well-recognized and established protein families with an undeniable presence in LECA. The actin family also includes a large group of ARPs that possibly took over functions of one ancestral actin. Likewise, the tubulin family has many subfamilies, whose members are often involved in the organization of MTOCs. The situation is quite distinct in the case of proteins forming the intermediate filaments. While some IF-like protein families appear to be quite widespread, particularly proteins with similarity to SF-assemblin, some are restricted to a particular lineage and connected to a specific cytoskeletal structure. Mutual relationships of IF-like proteins are often unclear. This might, however, be resolved by their identification in the ever-growing number of available genomes and more thorough studies.

The study of the proteins that make up these cytoskeletal structures might, in turn, shed light on the process of their emergence. Currently, the protein composition of many striated fibers in protist cells still awaits characterization. IF-like proteins are proposed as one of the main components of such structures. Therefore, we would like to investigate the presence of IF-like proteins in IF-like fibers in metamonads such as *Monocercomonoides exilis* and *Paratrimastix pyriformis* in the future.

7 References

Al-Khattaf, F. S., Tremp, A. Z., & Dessens, J. T. (2015). Plasmodium alveolins possess distinct but structurally and functionally related multi-repeat domains. *Parasitology Research*. https://doi.org/10.1007/s00436-014-4226-9

Alonso, R. A., & Peattie, D. A. (1992). Nucleotide sequence of a second alpha giardin gene and molecular analysis of the alpha giardin genes and transcripts in Giardia lamblia. *Molecular and Biochemical Parasitology*. https://doi.org/10.1016/0166-6851(92)90247-H

Anderson-White, B. R., Ivey, F. D., Cheng, K., Szatanek, T., Lorestani, A., Beckers, C. J., Ferguson, D. J. P., Sahoo, N., & Gubbels, M. J. (2011). A family of intermediate filament-like proteins is sequentially assembled into the cytoskeleton of Toxoplasma gondii. *Cellular Microbiology*, *13*(1), 18–31. https://doi.org/10.1111/j.1462-5822.2010.01514.x

Aubusson-Fleury, A., Bricheux, G., Damaj, R., Lemullois, M., Coffe, G., Donnadieu, F., Koll, F., Viguès, B., & Bouchard, P. (2013). Epiplasmins and Epiplasm in Paramecium: The Building of a Submembraneous Cytoskeleton. *Protist*, *164*(4), 451–469. https://doi.org/10.1016/j.protis.2013.04.003

Aury, J.-M., Jaillon, O., Duret, L., Noel, B., Jubin, C., Porcel, B. M., Ségurens, B., Daubin, V., Anthouard, V., Aiach, N., Arnaiz, O., Billaut, A., Beisson, J., Blanc, I., Bouhouche, K., Câmara, F., Duharcourt, S., Guigo, R., Gogendeau, D., … Wincker, P. (2006). Global trends of whole-genome duplications revealed by the ciliate Paramecium tetraurelia. *Nature*, *444*(7116), 171–178. https://doi.org/10.1038/nature05230

Bae, S. S., Kim, J., Kim, T. S., Yong, T. S., & Park, S. J. (2009). Giardia lamblia: Immunogenicity and intracellular distribution of GHSP-115, a member of the Giardia head-stalk family of proteins. *Experimental Parasitology*. https://doi.org/10.1016/j.exppara.2009.01.005

Bassen, D. M., Hou, Y., Bowser, S. S., & Banavali, N. K. (2016). Maintenance of electrostatic stabilization in altered tubulin lateral contacts may facilitate formation of helical filaments in foraminifera. *Scientific Reports*, *6*(1), 31723. https://doi.org/10.1038/srep31723

Batsios, P., Peter, T., Baumann, O., Stick, R., Meyer, I., & Gräf, R. (2012). A lamin in lower eukaryotes? *Nucleus (United States)*, *3*(3). https://doi.org/10.4161/nucl.20149

Bauer, B., Engelbrecht, S., Bakker-Grunwald, T., & Scholze, H. (1999). Functional identification of Î±1-giardin as an annexin of Giardia lamblia. *FEMS Microbiology Letters*, *173*(1), 147–153. https://doi.org/10.1111/j.1574-6968.1999.tb13496.x

Baum, J., Papenfuss, A. T., Baum, B., Speed, T. P., & Cowman, A. F. (2006). Regulation of apicomplexan actin-based motility. *Nature Reviews Microbiology*. https://doi.org/10.1038/nrmicro1465

Benchimol, M. (2004). Trichomonads under Microscopy. *Microscopy and Microanalysis*, *10*(5), 528–550. https://doi.org/10.1017/S1431927604040905

Bouchard, P., Chomilier, J., Ravet, V., Mornon, J. P., & Viguès, B. (2001). Molecular characterization of the major membrane skeletal protein in the ciliate Tetrahymena pyriformis suggests n-plication of an early evolutionary intermediate filament protein subdomain. *Journal of Cell Science*.

Brimmer, A., & Weber, K. (2000). The cDNA sequences of three tetrins, the structural proteins of the Tetrahymena oral filaments, show that they are novel cytoskeletal proteins. *Protist*. https://doi.org/10.1078/1434-4610-00017

Brugerolle, G., & Viscogliosi, E. (1994). Organization and composition of the striated roots supporting the Golgi apparatus, the so-called parabasal apparatus, in parabasalid flagellates. *Biology of the Cell*, *81*(3), 277–285. https://doi.org/10.1016/0248-4900(94)90010-8

Campanati, L., Troester, H., Monteiro-Leal, L. H., Spring, H., Trendelenburg, M. F., & De Souza, W. (2003). Tubulin diversity in trophozoites of Giardia lamblia. In *Histochemistry and Cell Biology* (Vol. 119, Issue 4, pp. 323–331). https://doi.org/10.1007/s00418-003-0517-4

Carvalho-Santos, Z., Azimzadeh, J., Pereira-Leal, J. B., & Bettencourt-Dias, M. (2011). Tracing the origins of centrioles, cilia, and flagella. *The Journal of Cell Biology*, *194*(2), 165–175. https://doi.org/10.1083/jcb.201011152

Cavalier-Smith, T. (2017). Euglenoid pellicle morphogenesis and evolution in light of comparative ultrastructure and trypanosomatid biology : Semi-conservative microtubule / strip duplication , strip shaping and transformation. *European Journal of Protistology*, *61*, 137–179. https://doi.org/10.1016/j.ejop.2017.09.002

Čepička, I., Dolan, M. F., & Gile, G. H. (2017). Parabasalia. In *Handbook of the Protists* (pp. 1175–1218). Springer International Publishing. https://doi.org/10.1007/978-3-319-28149-0_9

Chang, P., & Stearns, T. (2000). δ-Tubulin and ε-tubulin: two new human centrosomal tubulins reveal new aspects of centrosome structure and function. *Nature Cell Biology*, *2*(1), 30–35. https://doi.org/10.1038/71350

Clerot, J.-C., Iftode, F., Budin, K., Jeanmarie-Wolf, R., Coffe, G., & Fleury-Aubosson, A. (2001). Fine Oral Filaments in Paramecium: a Biochemical and Immunological Analysis. *The Journal of Eukaryotic Microbiology*, *48*(2), 234–245. https://doi.org/10.1111/j.1550-7408.2001.tb00310.x

Coffe, G., Caer, J. P. Le, Lima, O., & Adoutte, A. (1996). Purification, in vitro reassembly, preliminary sequence analysis of epiplasmins, the major constituent of the membrane skeleton of paramecium. *Cell Motility and the Cytoskeleton*.

Cohen, M., Gruenbaum, Y., Lee, K. K., & Wilson, K. L. (2001). Transcriptional repression, apoptosis, human disease and the functional evolution of the nuclear lamina. *Trends in Biochemical Sciences*, *26*(1), 41–47. https://doi.org/10.1016/S0968-0004(00)01727-8

Crossley, R., & Holberton, D. (1985). Assembly of 2.5 nm filaments from giardin, a protein associated with cytoskeletal microtubules in Giardia. In *Journal of Cell Science: Vol. VOL. 78* (pp. 205–231).

Crossley, R., & Holberton, D. V. (1983). Characterization of proteins from the cytoskeleton of Giardia lamblia. In *Journal of Cell Science: Vol. Vol. 59* (pp. 81–103).

Damaj, R., Pomel, S., Bricheux, G., Coffe, G., Viguès, B., Ravet, V., & Bouchard, P. (2009). Cross-study analysis of genomic data defines the ciliate multigenic epiplasmin family: strategies for functional analysis in Paramecium tetraurelia. *BMC Evolutionary Biology*, *9*(1), 125. https://doi.org/10.1186/1471-2148-9-125

Dawson, S. C. (2010). An insider's guide to the microtubule cytoskeleton of Giardia. *Cellular Microbiology*, *12*(5), 588–598. https://doi.org/10.1111/j.1462-5822.2010.01458.x

de Andrade Rosa, I., Caruso, M. B., de Oliveira Santos, E., Gonzaga, L., Zingali, R. B., de Vasconcelos, A. T. R., de Souza, W., & Benchimol, M. (2017). The costa of trichomonads: A complex macromolecular cytoskeleton structure made of uncommon proteins. *Biology of the Cell*. https://doi.org/10.1111/boc.201600050

Desai, A., & Mitchison, T. J. (1997). MICROTUBULE POLYMERIZATION DYNAMICS. *Annual Review of Cell and Developmental Biology*, *13*(1), 83–117. https://doi.org/10.1146/annurev.cellbio.13.1.83

Dittmer, T., & Misteli, T. (2011). The lamin protein family. *Genome Biology*, *12*(5). https://doi.org/10.1186/gb-2011-12-5-222

Dress, V. M., Yi, H., Musal, M. R., & Williams, N. E. (1992). Tetrin polypeptides are colocalized in the cortex of Tetrahymena. *Journal of Structural Biology*, *108*(3), 187–194. https://doi.org/10.1016/1047-8477(92)90018-6

DuBois, K. N., Alsford, S., Holden, J. M., Buisson, J., Swiderski, M., Bart, J. M., Ratushny, A. V., Wan, Y., Bastin, P., Barry, J. D., Navarro, M., Horn, D., Aitchison, J. D., Rout, M. P., & Field, M. C. (2012). NUP-1 is a large coiled-coil nucleoskeletal protein in trypanosomes with lamin-like functions. In *PLoS Biology* (Vol. 10, Issue 3). https://doi.org/10.1371/journal.pbio.1001287

Dupuis-Williams, P., Fleury-Aubusson, A., De Loubresse, N. G., Geoffroy, H., Vayssié, L., Galvani, A., Espigat, A., & Rossier, J. (2002). Functional role of ε-tubulin in the assembly of the centriolar microtubule scaffold. *Journal of Cell Biology*, *158*(7), 1183–1193. https://doi.org/10.1083/jcb.200205028

Dutcher, S. K. (1995). *Chapter 45 Purification of Basal Bodies and Basal Body Complexes from Chlamydomonas reinhardtii* (pp. 323–334). https://doi.org/10.1016/S0091-679X(08)60826-2

Dutcher, S. K. (2001a). Motile organelles: The importance of specific tubulin isoforms. *Current Biology*, *11*(11), 419–422. https://doi.org/10.1016/S0960-9822(01)00250-0

Dutcher, S. K. (2001b). The tubulin fraternity: Alpha to eta. *Current Opinion in Cell Biology*, *13*(1), 49–54. https://doi.org/10.1016/S0955-0674(00)00173-3

Dutcher, S. K. (2003). Long-lost relatives reappear: Identification of new members of the tubulin superfamily. *Current Opinion in Microbiology*, *6*(6), 634–640. https://doi.org/10.1016/j.mib.2003.10.016

Dutcher, S. K., Morrissette, N. S., Preble, A. M., Rackley, C., & Stanga, J. (2002). E-Tubulin Is an Essential Component of the Centriole. In *Molecular Biology of the Cell* (Vol. 13, Issue 11, pp. 3859–3869). https://doi.org/10.1091/mbc.E02-04-0205

Dutcher, S. K., & O'Toole, E. T. (2016). The basal bodies of Chlamydomonas reinhardtii. In *Cilia* (Vol. 5, Issue 1). https://doi.org/10.1186/s13630-016-0039-z

Dutcher, S. K., & Trabuco, E. C. (1998). The UNI3 Gene Is Required for Assembly of Basal Bodies of Chlamydomonas and Encodes δ-Tubulin, a New Member of the Tubulin Superfamily. *Molecular Biology of the Cell*, *9*(6), 1293–1308. https://doi.org/10.1091/mbc.9.6.1293

El-Haddad, H., Przyborski, J. M., Kraft, L. G. K., Mcfadden, G. I., Waller, R. F., & Gould, S. B. (2013). *Characterization of TtALV2, an Essential Charged Repeat Motif Protein of the Tetrahymena thermophila Membrane Skeleton*. https://doi.org/10.1128/EC.00050-13

Elmendorf, H. G., Dawson, S. C., & McCaffery, J. M. (2003). The cytoskeleton of Giardia lamblia. In *International Journal for Parasitology* (Vol. 33, Issue 1, pp. 3–28). https://doi.org/10.1016/S0020-7519(02)00228-X

Elmendorf, H. G., Rohrer, S. C., Khoury, R. S., Bouttenot, R. E., & Nash, T. E. (2005). Examination of a novel head-stalk protein family in Giardia lamblia characterised by the pairing of ankyrin repeats and coiled-coil domains. *International Journal for Parasitology*. https://doi.org/10.1016/j.ijpara.2005.03.009

Elmendorf, H. G., Singer, S. M., Pierce, J., Cowan, J., & Nash, T. E. (2001). Initiator and upstream elements in the α2-tubulin promoter of Giardia lamblia. *Molecular and Biochemical Parasitology*, *113*(1), 157–169. https://doi.org/10.1016/S0166-6851(01)00211-0

Feliziani, C., Merino, M. C., Rivero, M. R., Hellman, U., Pistoresi-Palencia, M. C., & Rápolo, A. S. (2011). Immunodominant proteins α-1 giardin and β-giardin are expressed in both assemblages A and B of Giardia lamblia. *BMC Microbiology*. https://doi.org/10.1186/1471-2180-11-233

Findeisen, P., Mühlhausen, S., Dempewolf, S., Hertzog, J., Zietlow, A., Carlomagno, T., & Kollmar, M. (2014). Six Subgroups and Extensive Recent Duplications Characterize the Evolution of the Eukaryotic Tubulin Protein

Family. *Genome Biology and Evolution*, *6*(9), 2274–2288. https://doi.org/10.1093/gbe/evu187

Fleury-Aubusson, A. (2003). "Novel cytoskeletal proteins in protists": Introductory remarks. *Journal of Eukaryotic Microbiology*, *50*(1), 3–8. https://doi.org/10.1111/j.1550-7408.2003.tb00099.x

Francia, M. E., Jordan, C. N., Patel, J. D., Sheiner, L., Demerly, J. L., Fellows, J. D., de Leon, J. C., Morrissette, N. S., Dubremetz, J. F., & Striepen, B. (2012). Cell Division in Apicomplexan Parasites Is Organized by a Homolog of the Striated Rootlet Fiber of Algal Flagella. In *PLoS Biology* (Vol. 10, Issue 12). https://doi.org/10.1371/journal.pbio.1001444

Fromherz, S., Giddings, T. H., Gomez-Ospina, N., & Dutcher, S. K. (2004). Mutations in α-tubulin promote basal body maturation and flagellar assembly in the absence of δ-tubulin. In *Journal of Cell Science* (Vol. 117, Issue 2, pp. 303–314). https://doi.org/10.1242/jcs.00859

Fuchs, E., & Weber, K. (1994). INTERMEDIA TE FILAMENTS : Structure , Dynamics , Function ,. *Proteins*.

Garreau de Loubresse, N., Ruiz, F., Beisson, J., & Klotz, C. (2001). Role of delta-tubulin and the C-tubule in assembly of Paramecium basal bodies. In *BMC Cell Biology* (Vol. 2). https://doi.org/10.1186/1471-2121-2-4

Gerke, V., & Moss, S. E. (2002). Annexins: From Structure to Function. *Physiological Reviews*, *82*(2), 331–371. https://doi.org/10.1152/physrev.00030.2001

Gómez de León, C. T., Díaz Martín, R. D., Mendoza Hernández, G., González Pozos, S., Ambrosio, J. R., & Mondragón Flores, R. (2014). Proteomic characterization of the subpellicular cytoskeleton of Toxoplasma gondii tachyzoites. *Journal of Proteomics*, *111*, 86–99. https://doi.org/10.1016/j.jprot.2014.03.008

Goodson, H. V., & Hawse, W. F. (2002). Molecular evolution of the actin family. In *Journal of Cell Science* (Vol. 115, Issue 13, pp. 2619–2622).

Gould, S. B., Kraft, L. G. K., Van Dooren, G. G., Goodman, C. D., Ford, K. L., Cassin, A. M., Bacic, A., McFadden, G. I., & Waller, R. F. (2011). Ciliate pellicular proteome identifies novel protein families with characteristic repeat motifs that are common to alveolates. *Molecular Biology and Evolution*. https://doi.org/10.1093/molbev/msq321

Gould, S. B., Tham, W. H., Cowman, A. F., McFadden, G. I., & Waller, R. F. (2008). Alveolins, a new family of cortical proteins that define the protist infrakingdom Alveolata. *Molecular Biology and Evolution*. https://doi.org/10.1093/molbev/msn070

Gubbels, M. J., Wieffer, M., & Striepen, B. (2004). Fluorescent protein tagging in Toxoplasma gondii: Identification of a novel inner membrane complex component conserved among Apicomplexa. *Molecular and Biochemical Parasitology*, *137*(1), 99–110. https://doi.org/10.1016/j.molbiopara.2004.05.007

Hagen, K. D., Hirakawa, M. P., House, S. A., Schwartz, C. L., Pham, J. K., Cipriano, M. J., de la Torre, M. J., Sek, A. C., Du, G., Forsythe, B. M., & Dawson, S. C. (2011). Novel structural components of the ventral disc and lateral crest in giardia intestinalis. *PLoS Neglected Tropical Diseases*. https://doi.org/10.1371/journal.pntd.0001442

Harper, J. D. I., Thuet, J., Lechtreck, K. F., & Hardham, A. R. (2009). Proteins related to green algal striated fiber assemblin are present in stramenopiles and alveolates. In *Protoplasma* (Vol. 236, Issues 1–4, pp. 97–101). https://doi.org/10.1007/s00709-009-0041-z

Hattier, T., Andrulis, E. D., & Tartakoff, A. M. (2007). Immobility, inheritance and plasticity of shape of the yeast nucleus. *BMC Cell Biology*, *8*. https://doi.org/10.1186/1471-2121-8-47

Herrmann, H., Strelkov, S. V., Burkhard, P., & Aebi, U. (2009). Intermediate filaments: Primary determinants of cell architecture and plasticity. In *Journal of Clinical Investigation*. https://doi.org/10.1172/JCI38214

Holberton, D., Baker, D. A., & Marshall, J. (1988). Segmented α-helical coiled-coil structure of the protein giardin from the Giardia cytoskeleton. In *Journal of Molecular Biology* (Vol. 204, Issue 3, pp. 789–795). https://doi.org/10.1016/0022-2836(88)90370-1

Honts, J. E., & Williams, N. E. (1990). Tetrins: polypeptides that form bundled filaments in Tetrahymena. *Journal of Cell Science*, *96*(2), 293–302.

Honts, J. E., & Williams, N. E. (2003). Novel cytoskeletal proteins in the cortex of Tetrahymena. *Journal of Eukaryotic Microbiology*, *50*(1), 9–14. https://doi.org/10.1111/j.1550-7408.2003.tb00100.x

Horio, T., Uzawa, S., Jung, M. K., Oakley, B. R., Tanaka, K., & Yanagida, M. (1991). The fission yeast γ-tubulin is essential for mitosis and is localized at microtubule organizing centres. In *Journal of Cell Science* (Vol. 99, Issue 4, pp. 693–700).

Hu, K., Johnson, J., Florens, L., Fraunholz, M., Suravajjala, S., DiLullo, C., Yates, J., Roos, D. S., & Murray, J. M. (2006). Cytoskeletal components of an invasion machine - The apical complex of Toxoplasma gondii. In *PLoS Pathogens* (Vol. 2, Issue 2, pp. 0121–0138). https://doi.org/10.1371/journal.ppat.0020013

Hu, K., Roos, D. S., & Murray, J. M. (2002). A novel polymer of tubulin forms the conoid of Toxoplasma gondii. *Journal of Cell Biology*, *156*(6), 1039–1050. https://doi.org/10.1083/jcb.200112086

Huttenlauch, I. (1995). Major epiplasmic proteins of ciliates are articulins: cloning, recombinant expression, and structural characterization. *The Journal of Cell Biology*, *130*(6), 1401–1412. https://doi.org/10.1083/jcb.130.6.1401

Huttenlauch, I., Peck, R. K., Plessmann, U., Weber, K., & Stick, R. (1998). Characterisation of two articulins, the major epiplasmic proteins comprising the membrane skeleton of the ciliate Pseudomicrothorax. *Journal of Cell*

Science.

Inclán, Y. F., & Nogales, E. (2001). Structural models for the self-assembly and microtubule interactions of gamma-, delta- and epsilon-tubulin. *Journal of Cell Science*, *114*(Pt 2), 413–422. http://www.ncbi.nlm.nih.gov/pubmed/11148142

Jenkins, M. C., O'Brien, C. N., Murphy, C., Schwarz, R., Miska, K., Rosenthal, B., & Trout, J. M. (2009). Antibodies to the Ventral Disc Protein δ-giardin Prevent in Vitro Binding of Giardia lamblia Trophozoites. *Journal of Parasitology*, *95*(4), 895–899. https://doi.org/10.1645/ge-1851r.1

Joachimiak, E., Pucciarelli, S., Barchetta, S., Ballarini, P., Kaczanowska, J., & Miceli, C. (2007). Cell Cycle-dependent Expression of γ-Tubulin in the Amicronuclear Ciliate Tetrahymena pyriformis. *Protist*, *158*(1), 39–50. https://doi.org/10.1016/j.protis.2006.08.001

Jones, L. J. F., Carballido-López, R., & Errington, J. (2001). Control of cell shape in bacteria: Helical, actin-like filaments in Bacillus subtilis. *Cell*, *104*(6), 913–922. https://doi.org/10.1016/S0092-8674(01)00287-2

Kaczanowska, J., Iftode, F., Coffe, G., Prajer, M., Kosciuszko, H., & Adoutte, A. (1996). The protein kinase inhibitor 6-dimethylaminopurine does not inhibit micronuclear mitosis, but impairs the rearrangement of cytoplasmic MTOCs and execution of cytokinesis in the ciliate Paramecium during transition to interphase. *European Journal of Protistology*, *32*(1), 2–17. https://doi.org/10.1016/S0932-4739(96)80034-4

Karnkowska, A., Treitli, S. C., Brzoň, O., Novák, L., Vacek, V., Soukal, P., Barlow, L. D., Herman, E. K., Pipaliya, S. V, Pánek, T., Žihala, D., Petrželková, R., Butenko, A., Eme, L., Stairs, C. W., Roger, A. J., Eliáš, M., Dacks, J. B., & Hampl, V. (2019). The Oxymonad Genome Displays Canonical Eukaryotic Complexity in the Absence of a Mitochondrion. *Molecular Biology and Evolution*, *36*(10), 2292–2312. https://doi.org/10.1093/molbev/msz147

Katris, N. J., van Dooren, G. G., McMillan, P. J., Hanssen, E., Tilley, L., & Waller, R. F. (2014). The Apical Complex Provides a Regulated Gateway for Secretion of Invasion Factors in Toxoplasma. In *PLoS Pathogens* (Vol. 10, Issue 4). https://doi.org/10.1371/journal.ppat.1004074

Khater, E. I., Sinden, R. E., & Dessens, J. T. (2004). A malaria membrane skeletal protein is essential for normal morphogenesis, motility, and infectivity of sporozoites. *Journal of Cell Biology*, *167*(3), 425–432. https://doi.org/10.1083/jcb.200406068

Kim, S., Bachvaroff, T. R., Handy, S. M., & Delwiche, C. F. (2011). Dynamics of actin evolution in dinoflagellates. *Molecular Biology and Evolution*, *28*(4), 1469–1480. https://doi.org/10.1093/molbev/msq332

Kloetzel, J. A. (1991). Identification and Properties of Plateins , Major Proteins in the Cortical Alveolar Plates of Euplotes 1. *The Journal of Protozoology*, *38*(4), 392–401. https://doi.org/10.1111/j.1550-7408.1991.tb01376.x

Kloetzel, J. A., Baroin-Tourancheau, A., Miceli, C., Barchetta, S., Farmar, J., Banerjee, D., & Fleury-Aubusson, A. (2003a). Cytoskeletal proteins with N-terminal signal peptides: Plateins in the ciliate Euplotes define a new family of articulins. In *Journal of Cell Science*. https://doi.org/10.1242/jcs.00333

Kloetzel, J. A., Baroin-Tourancheau, A., Miceli, C., Barchetta, S., Farmar, J., Banerjee, D., & Fleury-Aubusson, A. (2003b). Plateins: A novel family of signal peptide-containing articulins in euplotid ciliates. *Journal of Eukaryotic Microbiology*. https://doi.org/10.1111/j.1550-7408.2003.tb00102.x

Klotz, C., Ruiz, F., De Loubresse, N. G., Wright, M., Dupuis-Williams, P., & Beisson, J. (2003). Gamma-tubulin and MTOCs in Paramecium. *Protist*, *154*(2), 193–209. https://doi.org/10.1078/143446103322166509

Kollman, J. M., Zelter, A., Muller, E. G. D., Fox, B., Rice, L. M., Davis, T. N., Agard, D. a, & Labeling, G. (2008). The Structure of the γ-Tubulin Small Complex : Microtubule Nucleation. In *Molecular Biology of the Cell* (Vol. 19, Issue January, pp. 207–215). https://doi.org/10.1091/mbc.E07

Kollmar, M. (2015). Polyphyly of nuclear lamin genes indicates an early eukaryotic origin of the metazoan-type intermediate filament proteins. *Scientific Reports*. https://doi.org/10.1038/srep10652

Koreny, L., & Field, M. C. (2016). Ancient eukaryotic origin and evolutionary plasticity of nuclear lamina. *Genome Biology and Evolution*. https://doi.org/10.1093/gbe/evw087

Krüger, A., Batsios, P., Baumann, O., Luckert, E., Schwarz, H., Stick, R., Meyer, I., & Gräf, R. (2012). Characterization of NE81, the first lamin-like nucleoskeleton protein in a unicellular organism. *Molecular Biology of the Cell*, *23*(2), 360–370. https://doi.org/10.1091/mbc.E11-07-0595

Larsen, R. A., Cusumano, C., Fujioka, A., Lim-Fong, G., Patterson, P., & Pogliano, J. (2007). Treadmilling of a prokaryotic tubulin-like protein, TubZ, required for plasmid stability in Bacillus thuringiensis. *Genes & Development*, *21*(11), 1340–1352. https://doi.org/10.1101/gad.1546107

Leander, B. S., Esson, H. J., & Breglia, S. A. (2007). *Macroevolution of complex cytoskeletal systems in euglenids*. *1*, 987–1000. https://doi.org/10.1002/bies.20645

Lechtreck, K. F. (1998). Analysis of striated fiber formation by recombinant SF-assemblin in vitro. *Journal of Molecular Biology*, *279*(2), 423–438. https://doi.org/10.1006/jmbi.1998.1763

Lechtreck, K. F. (2003). Striated fiber assemblin in apicomplexan parasites. *Molecular and Biochemical Parasitology*, *128*(1), 95–99. https://doi.org/10.1016/S0166-6851(03)00038-0

Lechtreck, K. F., Frins, S., Bilski, J., Teltenkötter, A., Weber, K., & Melkonian, M. (1996). The cruciated microtubule-associated fibers of the green alga Dunaliella bioculata consist of a 31 kDa SF-assemblin. In *Journal of Cell Science* (Vol. 109, Issue 4, pp. 827–835).

Lechtreck, K. F., & Melkonian, M. (1998). SF-assemblin, striated fibers, and segmented coiled coil proteins. *Cell Motility and the Cytoskeleton*, *41*(4), 289–296. https://doi.org/10.1002/(SICI)1097-0169(1998)41:4<289::AID-CM2>3.0.CO;2-1

Lechtreck, K. F., Rostmann, J., & Grunow, A. (2002). Analysis of Chlamydomonas SF-assemblin by GFP tagging and expression of antisense constructs. In *Journal of Cell Science* (Vol. 115, Issue 7, pp. 1511–1522).

Lechtreck, K. F., & Silflow, C. D. (1997). SF-assemblin in Chlamydomonas: Sequence conservation and localization during the cell cycle. *Cell Motility and the Cytoskeleton*, *36*(2), 190–201. https://doi.org/10.1002/(SICI)1097-0169(1997)36:2<190::AID-CM8>3.0.CO;2-D

Libusová, L., & Dráber, P. (2006). Multiple tubulin forms in ciliated protozoan Tetrahymena and Paramecium species. In *Protoplasma* (Vol. 227, Issues 2–4, pp. 65–76). https://doi.org/10.1007/s00709-005-0152-0

Long, S., Anthony, B., Drewry, L. L., & Sibley, L. D. (2017). A conserved ankyrin repeat-containing protein regulates conoid stability, motility and cell invasion in Toxoplasma gondii. In *Nature Communications* (Vol. 8, Issue 1). https://doi.org/10.1038/s41467-017-02341-2

Lourenço, D., Andrade, I., Terra, L., Guimarães, P., Zingali, R., & de Souza, W. (2012). Proteomic analysis of the ventral disc of Giardia lamblia. *BMC Research Notes*, *5*(1), 41. https://doi.org/10.1186/1756-0500-5-41

Ludueña, R. F. (1997). Multiple forms of tubulin: Different gene products and covalent modifications. *International Review of Cytology*, *178*, 207–275. https://doi.org/10.1016/s0074-7696(08)62138-5

Mann, T., & Beckers, C. (2001). Characterization of the subpellicular network, a filamentous.pdf. *Molecular & Biochemical Parasitology*, *115*, 257–268.

Mann, T., Gaskins, E., & Beckers, C. (2002). Proteolytic processing of TgIMC1 during maturation of the membrane skeleton of Toxoplasma gondii. *Journal of Biological Chemistry*, *277*(43), 41240–41246. https://doi.org/10.1074/jbc.M205056200

Marrs, J. A., & Bouck, G. B. (1992). The two major membrane skeletal proteins (articulins) of Euglena gracilis define a novel class of cytoskeletal proteins. *Journal of Cell Biology*. https://doi.org/10.1083/jcb.118.6.1465

Marshall, W. F., & Rosenbaum, J. L. (2003). Tubulin superfamily: Giving birth to triplets. *Current Biology*, *13*(2), 55–56. https://doi.org/10.1016/S0960-9822(02)01427-6

Marziale, F., Pucciarelli, S., Ballarini, P., Melki, R., Uzun, A., Ilyin, V. A., Detrich III, H. W., & Miceli, C. (2008). Different roles of two γ-tubulin isotypes in the cytoskeleton of the Antarctic ciliate Euplotes focardii. *FEBS Journal*, *275*(21), 5367–5382. https://doi.org/10.1111/j.1742-4658.2008.06666.x

Mason, J. M., & Arndt, K. M. (2004). Coiled Coil Domains: Stability, Specificity, and Biological Implications. *ChemBioChem*, *5*(2), 170–176. https://doi.org/10.1002/cbic.200300781

McKean, P., Vaughan, S., & Gull, K. (2001). The extended tubulin superfamily. *Journal of Cell Science*.

McLaughlin, N. B., & Buhse, H. E. (2004). Localization by indirect immunofluorescence of tetrin, actin, and centrin to the oral apparatus and buccal cavity of the macrostomal form of Tetrahymena vorax. *Journal of Eukaryotic Microbiology*, *51*(2), 253–257. https://doi.org/10.1111/j.1550-7408.2004.tb00556.x

Melcer, S., & Gruenbaum, Y. (2006). Nuclear morphology: When round kernels do the Charleston. *Current Biology*, *16*(6), 195–197. https://doi.org/10.1016/j.cub.2006.02.032

Melcer, S., Gruenbaum, Y., & Krohne, G. (2007). Invertebrate lamins. *Experimental Cell Research*, *313*(10), 2157–2166. https://doi.org/10.1016/j.yexcr.2007.03.004

Momany, M., Pan, F., & Malmberg, R. L. (2008). Evolution and Conserved Domains of the Septins. In *The Septins* (pp. 35–45). John Wiley & Sons, Ltd. https://doi.org/10.1002/9780470779705.ch2

Morgan, R. O., & Fernandéz, M.-P. (1995). Molecular phylogeny of annexins and identification of a primitive homologue in Giardia lamblia. *Molecular Biology and Evolution*. https://doi.org/10.1093/oxfordjournals.molbev.a040290

Muller, J., Oma, Y., Vallar, L., Friederich, E., Poch, O., & Winsor, B. (2005). Sequence and comparative genomic analysis of actin-related proteins. In *Molecular Biology of the Cell* (Vol. 16, Issue 12, pp. 5736–5748). https://doi.org/10.1091/mbc.E05-06-0508

Nishihama, R., Onishi, M., & Pringle, J. R. (2011). New insights into the phylogenetic distribution and evolutionary origins of the septins. *Biological Chemistry*, *392*(8–9), 681–687. https://doi.org/10.1515/BC.2011.086

Nogales, E. (2001). Structural Insights into Microtubule Function. *Annual Review of Biophysics and Biomolecular Structure*, *30*(1), 397–420. https://doi.org/10.1146/annurev.biophys.30.1.397

Nohria, A., Alonso, R. A., & Peattie, D. A. (1992). Identification and characterization of γ-giardin and the γ-giardin gene from Giardia lamblia. *Molecular and Biochemical Parasitology*. https://doi.org/10.1016/0166-6851(92)90151-9

Nohýnková, E., Dráber, P., Reischig, J., & Kulda, J. (2000). Localization of gamma-tubulin in interphase and mitotic cells of a unicellular eukaryote, Giardia intestinalis. *European Journal of Cell Biology*, *79*(6), 438–445. https://doi.org/10.1078/0171-9335-00066

O'Toole, E. T., Giddings, T. H., McIntosh, J. R., & Dutcher, S. K. (2003). Three-dimensional Organization of Basal Bodies from Wild-Type and δ-Tubulin Deletion Strains of Chlamydomonas reinhardtii. *Molecular Biology of the Cell*, *14*(7), 2999–3012. https://doi.org/10.1091/mbc.e02-11-0755

Oakley, B. R. (2000). An abundance of tubulins. *Trends in Cell Biology*, *10*(12), 537–542. https://doi.org/10.1016/S0962-8924(00)01857-2

Oakley, C. E., & Oakley, B. R. (1989). Identification of γ-tubulin, a new member of the tubulin superfamily encoded by mipA gene of Aspergillus nidulans. *Nature*, *338*(6217), 662–664. https://doi.org/10.1038/338662a0

Onishi, M., & Pringle, J. R. (2016). The nonopisthokont septins: How many there are, how little we know about them, and how we might learn more. In *Methods in Cell Biology* (Vol. 136, pp. 1–19). https://doi.org/10.1016/bs.mcb.2016.04.003

Palm, J. E. D., Weiland, M. E. -L., Griffiths, W. J., Ljungström, I., & Svärd, S. G. (2003). Identification of Immunoreactive Proteins during Acute Human Giardiasis. *The Journal of Infectious Diseases*, *187*(12), 1849–1859. https://doi.org/10.1086/375356

Palm, J. E. D., Weiland, M., McArthur, A. G., Winiecka-Krusnell, J., Cipriano, M. J., Birkeland, S. R., Pacocha, S. E., Davids, B., Gillin, F., Linder, E., & Svärd, S. (2005). Developmental changes in the adhesive disk during Giardia differentiation. *Molecular and Biochemical Parasitology*, *141*(2), 199–207. https://doi.org/10.1016/j.molbiopara.2005.03.005

Pastuglia, M., Azimzadeh, J., Goussot, M., Camilleri, C., Belcram, K., Evrard, J.-L., Schmit, A.-C., Guerche, P., & Bouchez, D. (2006). γ-Tubulin Is Essential for Microtubule Organization and Development in Arabidopsis. *The Plant Cell*, *18*(6), 1412–1425. https://doi.org/10.1105/tpc.105.039644

Patel, H., Lechtreck, K. F., Melkonian, M., & Mandelkow, E. (1992). Structure of striated microtubule-associated fibers of flagellar roots. Comparison of native and reconstituted states. In *Journal of Molecular Biology* (Vol. 227, Issue 3, pp. 698–710). https://doi.org/10.1016/0022-2836(92)90218-9

Peattie, D. A. (1990). The Giardins of Giardia lamblia: Genes and proteins with promise. In *Parasitology Today* (Vol. 6, Issue 2, pp. 52–56). https://doi.org/10.1016/0169-4758(90)90070-K

Peattie, D. A., Alonso, R. A., Hein, A., & Caulfield, J. P. (1989). Ultrastructural localization of giardins to the edges of disk microribbons of Giardia lamblia and the nucleotide and deduced protein sequence of alpha giardin -b. *Journal of Cell Biology*. https://doi.org/10.1083/jcb.109.5.2323

Peter, A., & Stick, R. (2015). Evolutionary aspects in intermediate filament proteins. *Current Opinion in Cell Biology*, *32*, 48–55. https://doi.org/10.1016/j.ceb.2014.12.009

Peter, M., Nakagawa, J., Dorée, M., Labbé, J. C., & Nigg, E. A. (1990). In vitro disassembly of the nuclear lamina and M phase-specific phosphorylation of lamins by cdc2 kinase. In *Cell* (Vol. 61, Issue 4, pp. 591–602). https://doi.org/10.1016/0092-8674(90)90471-P

Pfannenschmid, F., Wimmer, V. C., Rios, R. M., Geimer, S., Kröckel, U., Leiherer, A., Haller, K., Nemcová, Y., & Mages, W. (2003). Chlamydomonas DIP13 and human NA14: A new class of proteins associated with microtubule structures is involved in cell division. In *Journal of Cell Science* (Vol. 116, Issue 8, pp. 1449–1462). https://doi.org/10.1242/jcs.00337

Pomel, S., Diogon, M., Bouchard, P., Pradel, L., Ravet, V., Coffe, G., & Viguès, B. (2006). The membrane skeleton in Paramecium: Molecular characterization of a novel epiplasmin family and preliminary GFP expression results. *Protist*, *157*(1), 61–75. https://doi.org/10.1016/j.protis.2005.11.002

Portman, N., & Gull, K. (2010). The paraflagellar rod of kinetoplastid parasites: From structure to components and function. *International Journal for Parasitology*, *40*(2), 135–148. https://doi.org/10.1016/j.ijpara.2009.10.005

Preisner, H., Habicht, J., Garg, S. G., & Gould, S. B. (2018). Intermediate filament protein evolution and protists. *Cytoskeleton*, *75*(6), 231–243. https://doi.org/10.1002/cm.21443

Preisner, H., Karin, E. L., Poschmann, G., Stühler, K., Pupko, T., & Gould, S. B. (2016). The Cytoskeleton of Parabasalian Parasites Comprises Proteins that Share Properties Common to Intermediate Filament Proteins. In *Protist* (Vol. 167, Issue 6, pp. 526–543). https://doi.org/10.1016/j.protis.2016.09.001

Rose, A., Schraegle, S. J., Stahlberg, E. A., & Meier, I. (2005). Coiled-coil protein composition of 22 proteomes - Differences and common themes in subcellular infrastructure and traffic control. *BMC Evolutionary Biology*, *5*. https://doi.org/10.1186/1471-2148-5-66

Rosenbaum, J. (2000). Cytoskeleton: Functions for tubulin modifications at last. *Current Biology*, *10*(21), 801–803. https://doi.org/10.1016/S0960-9822(00)00767-3

Rosiere, T. K. (1990). A 39-kD plasma membrane protein (IP39) is an anchor for the unusual membrane skeleton of Euglena gracilis. *The Journal of Cell Biology*, *110*(4), 1077–1088. https://doi.org/10.1083/jcb.110.4.1077

Ross, I., Clarissa, C., Giddings, T. H., & Winey, M. (2013). E-tubulin is essential in Tetrahymena thermophila for the assembly and stability of basal bodies. *Journal of Cell Science*, *126*(15), 3441–3451. https://doi.org/10.1242/jcs.128694

Rout, M. P., Obado, S. O., Schenkman, S., & Field, M. C. (2017). Specialising the parasite nucleus: Pores, lamins, chromatin, and diversity. *PLoS Pathogens*, *13*(3). https://doi.org/10.1371/journal.ppat.1006170

Ruiz, F., Beisson, J., Rossier, J., & Dupuis-Williams, P. (1999). Basal body duplication in Paramecium requires γ-tubulin. *Current Biology*, *9*(1), 43–46. https://doi.org/10.1016/S0960-9822(99)80045-1

Ruiz, F., Dupuis-Williams, P., Klotz, C., Forquignon, F., Bergdoll, M., Beisson, J., & Koll, F. (2004). Genetic Evidence for Interaction between η- and β-Tubulins. *Eukaryotic Cell*, *3*(1), 212–220. https://doi.org/10.1128/EC.3.1.212-220.2004

Ruiz, F., Krzywicka, A., Klotz, C., Keller, A. M., Cohen, J., Koll, F., Balavoine, G., & Beisson, J. (2000). The SM19 gene, required for duplication of basal bodies in Paramecium, encodes a novel tubulin, η-tubulin. *Current Biology*, *10*(22), 1451–1454. https://doi.org/10.1016/S0960-9822(00)00804-6

Salje, J., & Löwe, J. (2008). Bacterial actin: Architecture of the ParMRC plasmid DNA partitioning complex. *EMBO Journal*, *27*(16), 2230–2238. https://doi.org/10.1038/emboj.2008.152

Schlaeppi, K., Deflorin, J., & Seebeck, T. (1989). The major component of the paraflagellar rod of Trypanosoma brucei is a helical protein that is encoded by two identical, tandemly linked genes. *The Journal of Cell Biology*, *109*(4), 1695–1709. https://doi.org/10.1083/jcb.109.4.1695

Schmitz, S., Grainger, M., Howell, S., Calder, L. J., Gaeb, M., Pinder, J. C., Holder, A. A., & Veigel, C. (2005). Malaria parasite actin filaments are very short. *Journal of Molecular Biology*, *349*(1), 113–125. https://doi.org/10.1016/j.jmb.2005.03.056

Schoppmeier, J., Mages, W., & Lechtreck, K. F. (2005). GFP as a tool for the analysis of proteins in the flagellar basal apparatus of Chlamydomonas. *Cell Motility and the Cytoskeleton*, *61*(4), 189–200. https://doi.org/10.1002/cm.20074

Schüler, H., & Matuschewski, K. (2006). Plasmodium motility: Actin not actin' like actin. *Trends in Parasitology*, *22*(4), 146–147. https://doi.org/10.1016/j.pt.2006.02.005

Schüler, H., Mueller, A. K., & Matuschewski, K. (2005a). A Plasmodium actin-depolymerizing factor that binds exclusively to actin monomers. In *Molecular Biology of the Cell* (Vol. 16, Issue 9, pp. 4013–4023). https://doi.org/10.1091/mbc.E05-02-0086

Schüler, H., Mueller, A. K., & Matuschewski, K. (2005b). Unusual properties of Plasmodium falciparum actin: New insights into microfilament dynamics of apicomplexan parasites. *FEBS Letters*, *579*(3), 655–660. https://doi.org/10.1016/j.febslet.2004.12.037

Schwartz, C. L., Heumann, J. M., Dawson, S. C., & Hoenger, A. (2012). A Detailed, Hierarchical Study of Giardia lamblia's Ventral Disc Reveals Novel Microtubule-Associated Protein Complexes. In *PLoS ONE* (Vol. 7, Issue 9). https://doi.org/10.1371/journal.pone.0043783

Scott, V., Sherwin, T., & Gull, K. (1997). γ-tubulin in trypanosomes: Molecular characterisation and localisation to multiple and diverse microtubule organising centres. In *Journal of Cell Science* (Vol. 110, Issue 2, pp. 157–168).

Shang, Y., Li, B., & Gorovsky, M. A. (2002). Tetrahymena thermophila contains a conventional γ-tubulin that is differentially required for the maintenance of different microtubule-organizing centers. *Journal of Cell Biology*, *158*(7), 1195–1206. https://doi.org/10.1083/jcb.200205101

Shang, Y., Tsao, C.-C., & Gorovsky, M. A. (2005). Mutational analyses reveal a novel function of the nucleotide-binding domain of γ-tubulin in the regulation of basal body biogenesis. *Journal of Cell Biology*, *171*(6), 1035–1044. https://doi.org/10.1083/jcb.200508184

Stearns, T., Evans, L., & Kirschner, M. (1991). γ-Tubulin is a highly conserved component of the centrosome. *Cell*, *65*(5), 825–836. https://doi.org/10.1016/0092-8674(91)90390-K

Strelkov, S. V., Herrmann, H., & Aebi, U. (2003). *Molecular architecture of intermediate filaments*. https://doi.org/10.1002/bies.10246

Subota, I., Julkowska, D., Vincensini, L., Reeg, N., Buisson, J., Blisnick, T., Huet, D., Perrot, S., Santi-Rocca, J., Duchateau, M., Hourdel, V., Rousselle, J.-C., Cayet, N., Namane, A., Chamot-Rooke, J., & Bastin, P. (2014). Proteomic Analysis of Intact Flagella of Procyclic Trypanosoma brucei Cells Identifies Novel Flagellar Proteins with Unique Sub-localization and Dynamics. *Molecular & Cellular Proteomics*, *13*(7), 1769–1786. https://doi.org/10.1074/mcp.M113.033357

Sunkel, C. E., Gomes, R., Sampaio, P., Perdigão, J., & González, C. (1995). Gamma-tubulin is required for the structure and function of the microtubule organizing centre in Drosophila neuroblasts. *The EMBO Journal*, *14*(1), 28–36. https://doi.org/10.1002/j.1460-2075.1995.tb06972.x

Szkodowska, A., Müller, M. C. M., Linke, C., & Scholze, H. (2002). Annexin XXI (ANX21) of Giardia lamblia has sequence motifs uniquely shared by giardial annexins and is specifically localized in the flagella. *Journal of Biological Chemistry*, *277*(28), 25703–25706. https://doi.org/10.1074/jbc.M203260200

Talke, S., & Preisfeld, A. (2002). MOLECULAR EVOLUTION OF EUGLENOZOAN PARAXONEMAL ROD GENES par1 AND par2 COINCIDES WITH PHYLOGENETIC RECONSTRUCTION BASED ON SMALL SUBUNIT rDNA DATA1. *Journal of Phycology*, *38*(5), 995–1003. https://doi.org/10.1046/j.1529-8817.2002.02028.x

Tran, J. Q., De Leon, J. C., Li, C., Huynh, M. H., Beatty, W., & Morrissette, N. S. (2010). RNG1 is a late marker of the apical polar ring in Toxoplasma gondii. *Cytoskeleton*. https://doi.org/10.1002/cm.20469

Tremp, A. Z., & Dessens, J. T. (2011). Malaria IMC1 membrane skeleton proteins operate autonomously and participate in motility independently of cell shape. *Journal of Biological Chemistry*, *286*(7), 5383–5391. https://doi.org/10.1074/jbc.M110.187195

Tremp, A. Z., Khater, E. I., & Dessens, J. T. (2008). IMC1b is a putative membrane skeleton protein involved in cell shape, mechanical strength, motility, and infectivity of malaria ookinetes. *Journal of Biological Chemistry*, *283*(41), 27604–27611. https://doi.org/10.1074/jbc.M801302200

Turk, E., Wills, A. A., Kwon, T., Sedzinski, J., Wallingford, J. B., & Stearns, T. (2015). Zeta-tubulin is a member of a

conserved tubulin module and is a component of the centriolar basal foot in multiciliated cells. *Current Biology*, *25*(16), 2177–2183. https://doi.org/10.1016/j.cub.2015.06.063

Vahrmann, A., Šarić, M., Scholze, H., & Koebsch, I. (2008). α14-Giardin (annexin E1) is associated with tubulin in trophozoites of Giardia lamblia and forms local slubs in the flagella. *Parasitology Research*. https://doi.org/10.1007/s00436-007-0758-6

Vaughan, S., Attwood, T., Navarro, M., Scott, V., McKean, P., & Gull, K. (2000). New tubulins in protozoal parasites. *Current Biology*, *10*(7), R258–R259. https://doi.org/10.1016/S0960-9822(00)00414-0

Viscogliosi, E., & Brugerolle, G. (1994). Striated fibers in trichomonads: Costa proteins represent a new class of proteins forming striated roots. *Cell Motility and the Cytoskeleton*. https://doi.org/10.1002/cm.970290108

Volkmann, K., Pfander, C., Burstroem, C., Ahras, M., Goulding, D., Rayner, J. C., Frischknecht, F., Billker, O., & Brochet, M. (2012). The alveolin IMC1h is required for normal ookinete and sporozoite motility behaviour and host colonisation in plasmodium berghei. In *PLoS ONE* (Vol. 7, Issue 7). https://doi.org/10.1371/journal.pone.0041409

Votýpka, J., Modrý, D., Obornik, M., Šlapeta, J., & Lukeš, J. (2017). Apicomplexa. In *Handbook of the Protists: Second Edition*. https://doi.org/10.1007/978-3-319-28149-0_20

Ward, G. E., & Kirschner, M. W. (1990). Identification of cell cycle-regulated phosphorylation sites on nuclear lamin C. *Cell*, *61*(4), 561–577. https://doi.org/10.1016/0092-8674(90)90469-U

Weber, K., Geisler, N., Plessmann, U., Bremerich, A., Lechtreck, K. F., & Melkonian, M. (1993). SF-assemblin, the structural protein of the 2-nm filaments from striated microtubule associated fibers of algal flagellar roots, forms a segmented coiled coil. *Journal of Cell Biology*, *121*(4), 837–845. https://doi.org/10.1083/jcb.121.4.837

Wei, C. J., Tian, X. F., Adam, R. D., & Lu, S. Q. (2010). Giardia lamblia: Intracellular localization of alpha8-giardin. *Experimental Parasitology*, *126*(4), 489–496. https://doi.org/10.1016/j.exppara.2010.05.028

Weiland, M. E. L., McArthur, A. G., Morrison, H. G., Sogin, M. L., & Svärd, S. G. (2005). Annexin-like alpha giardins: A new cytoskeletal gene family in Giardia lamblia. *International Journal for Parasitology*, *35*(6), 617–626. https://doi.org/10.1016/j.ijpara.2004.12.009

Weiland, M. E. L., Palm, J. E. D., Griffiths, W. J., McCaffery, J. M., & Svärd, S. G. (2003). Characterisation of alpha-1 giardin: An immunodominant Giardia lamblia annexin with glycosaminoglycan-binding activity. *International Journal for Parasitology*, *33*(12), 1341–1351. https://doi.org/10.1016/S0020-7519(03)00201-7

Wen, J. (2000). The nuclear matrix of Euglena gracilis (Euglenophyta): A stage of nuclear matrix evolution? *Biology of the Cell*, *92*(2), 125–131. https://doi.org/10.1016/S0248-4900(00)89020-6

Wen, J., & Li, J. (1998). Nuclear matrix of the most primitive eukaryote Archezoa. In *Science in China, Series C: Life Sciences* (Vol. 41, Issue 5, pp. 479–487). https://doi.org/10.1007/BF02882885

Wenman, W. M., Meuser, R. U., Nyugen, Q., Kilani, R. T., El-Shewy, K., & Sherburne, R. (1993). Characterization of an immunodominant Giardia lamblia protein antigen related to alpha giardin. *Parasitology Research*, *79*(7), 587–592. https://doi.org/10.1007/BF00932243

Wickstead, B., & Gull, K. (2011). The evolution of the cytoskeleton. In *Journal of Cell Biology*. https://doi.org/10.1083/jcb.201102065

Williams, N. E. (2004). *The Epiplasm Gene EPCl Influences Cell Shape and Cortical Pattern in Tetrahymena thermophilal* (Vol. 5, Issue 2).

Williams, N. E., Honts, J. E., Lu, Q., Olson, C. L., & Moore, K. C. (1989). Identification and localization of major cortical proteins in the ciliated protozoan, Euplotes eurystomus. *Journal of Cell Science*, *92 (Pt 3)*, 433–439.

Włoga, D., Strzyzewska-Jówko, I., Gaertig, J., & Jerka-Dziadosz, M. (2008). Septins stabilize mitochondria in Tetrahymena thermophila. *Eukaryotic Cell*, *7*(8), 1373–1386. https://doi.org/10.1128/EC.00085-08

Yamazaki, T., Owari, S., Ota, S., Sumiya, N., Yamamoto, M., Watanabe, K., Nagumo, T., Miyamura, S., & Kawano, S. (2013). Localization and evolution of septins in algae. *Plant Journal*, *74*(4), 605–614. https://doi.org/10.1111/tpj.12147

Yu, I., Garnham, C. P., & Roll-Mecak, A. (2015). Writing and Reading the Tubulin Code. *Journal of Biological Chemistry*, *290*(28), 17163–17172. https://doi.org/10.1074/jbc.R115.637447